Introduction to metagenomic analysis

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Harvard University CFAR Workshop on
Metagenomics and Transcriptomics

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MiRiBA

BROAD INSTITUTE

http://huttenhower.sph.harvard.edu/biobakery

Huttenhower Lab Tools

Welcome to the official Huttenhower Tutorials wiki.

We now support `bioBakery`, a virtual environment platform that provides Huttenhower tools (already installed!). Please click on the button below for more information:

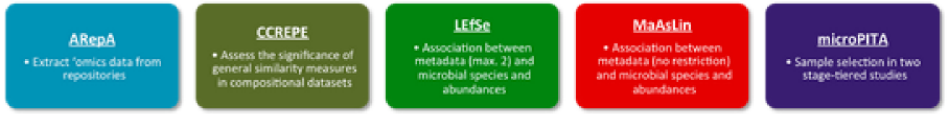
The wiki provides tutorials for Huttenhower tools, illustrating through demos how to use these tools on your datasets. Huttenhower tools can be divided under three main categories as shown below. Click on the tool for the corresponding tutorial.

Composition Analysis

These tools can determine the composition in terms of (i) microbial species and their associated abundances (MetaPhlAn) or (ii) genes and associated pathways (HUMAnN) in the dataset. Please click on the links below for detailed tutorials:

Statistical Analysis

These tools can determine the associations from the provided metadata information and microbial composition tables. Please click on the links below for detailed tutorials:

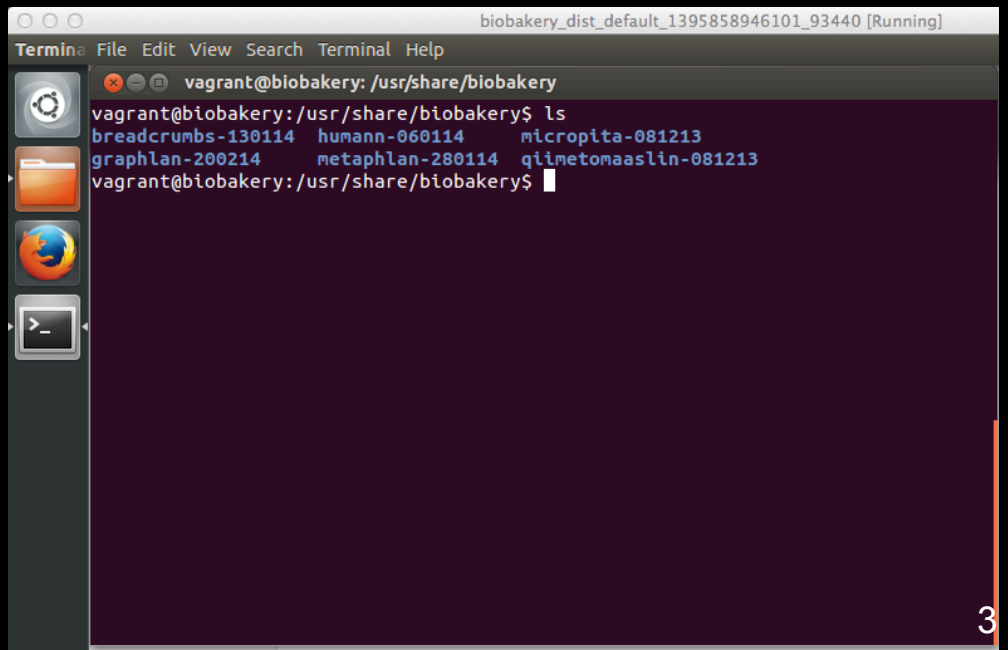


Visualization

These tools can help visualize taxonomical and phylogenetic information for (i) microbial composition/taxonomy data, (ii) outputs from MetaPhlAn, LEfSe, HUMAnN, MaAsLin. Please click on the link below for detailed tutorial:

The bioBakery: a next-generation environment for microbiome analyses

- Environment for meta'ome analysis
  - Shotgun metagenomes/transcriptomes
  - Taxonomic and functional profiling
  - Experimental design, statistical analysis
- Pre-built one-click environments to run:
  - On your laptop graphically
  - On a server remotely
  - On the cloud (Amazon)



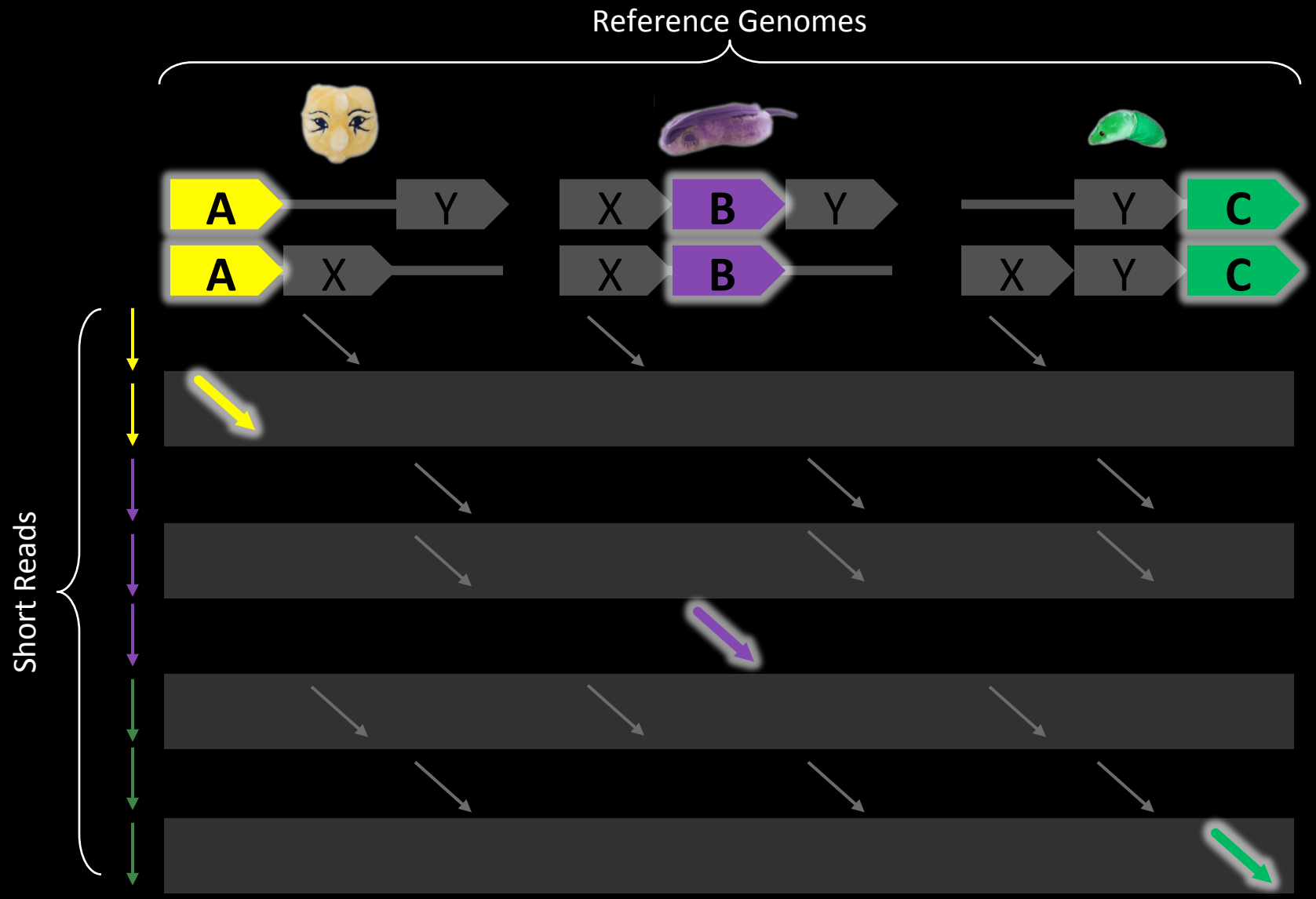
http://huttenhower.sph.harvard.edu/biobakery

The two big questions…

Who is there?
(taxonomic profiling)

What are they doing?
(functional profiling)

MetaPhlAn
Metagenomic Phylogenetic Analysis

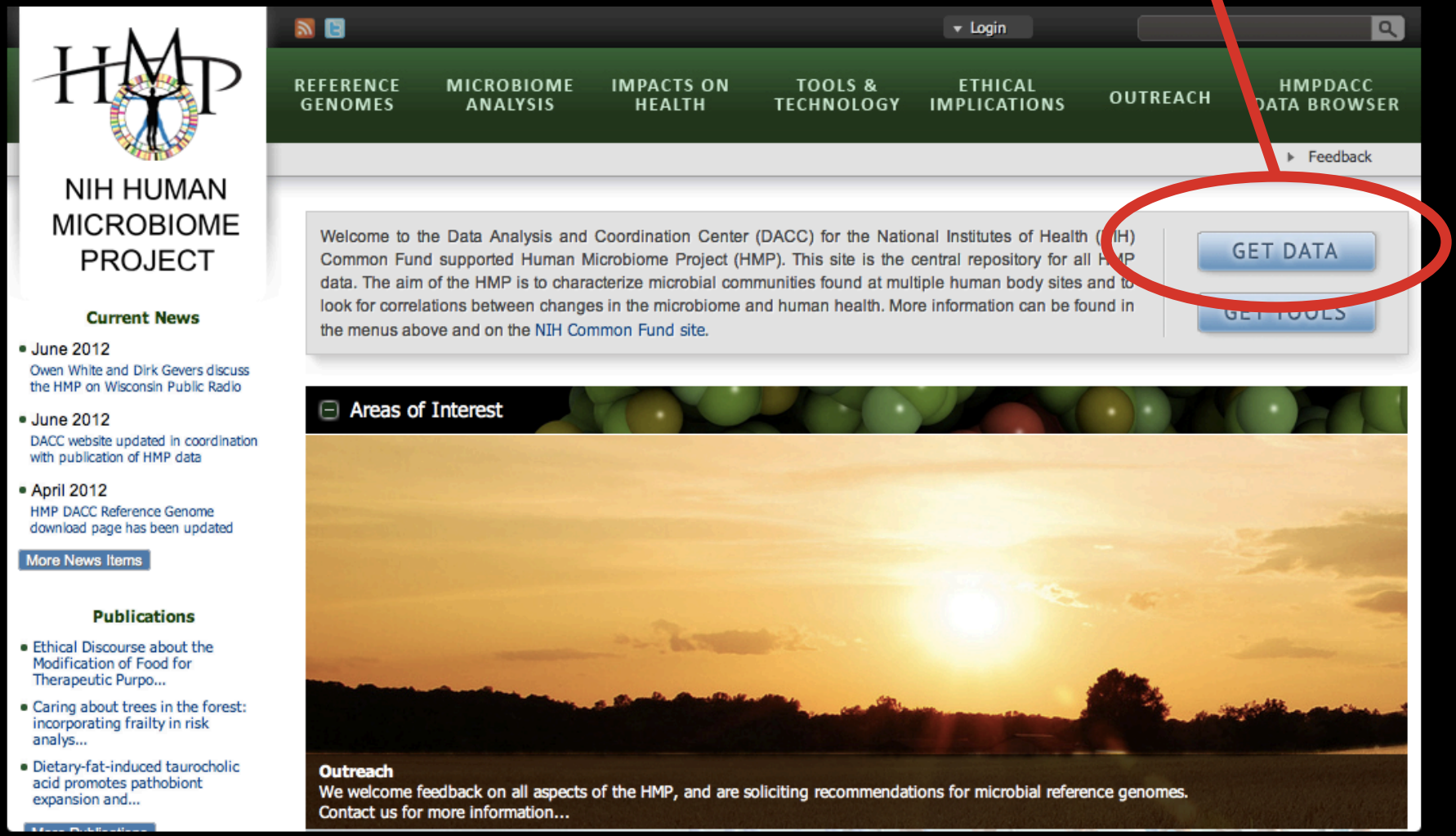


Some setup notes

- Slides with green titles or text include instructions not needed today, but useful for your own analyses
- Keep an eye out for red warnings of particular importance
- Command lines and program/file names appear in a `monospaced font`.
- Commands you should specifically copy/paste are in **`monospaced bold blue`**.

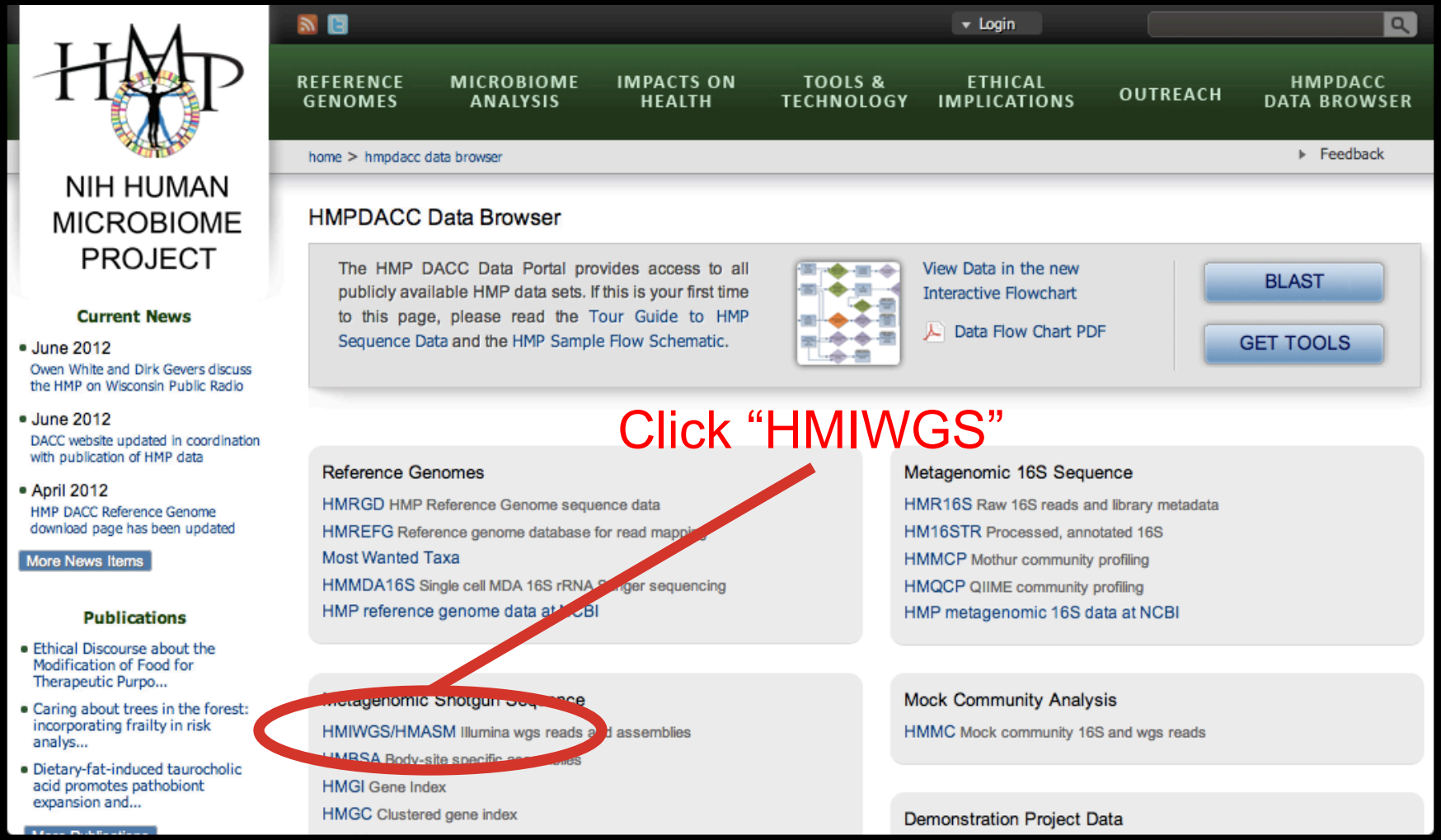
Getting some HMP data

- Go to http://hmpdacc.org



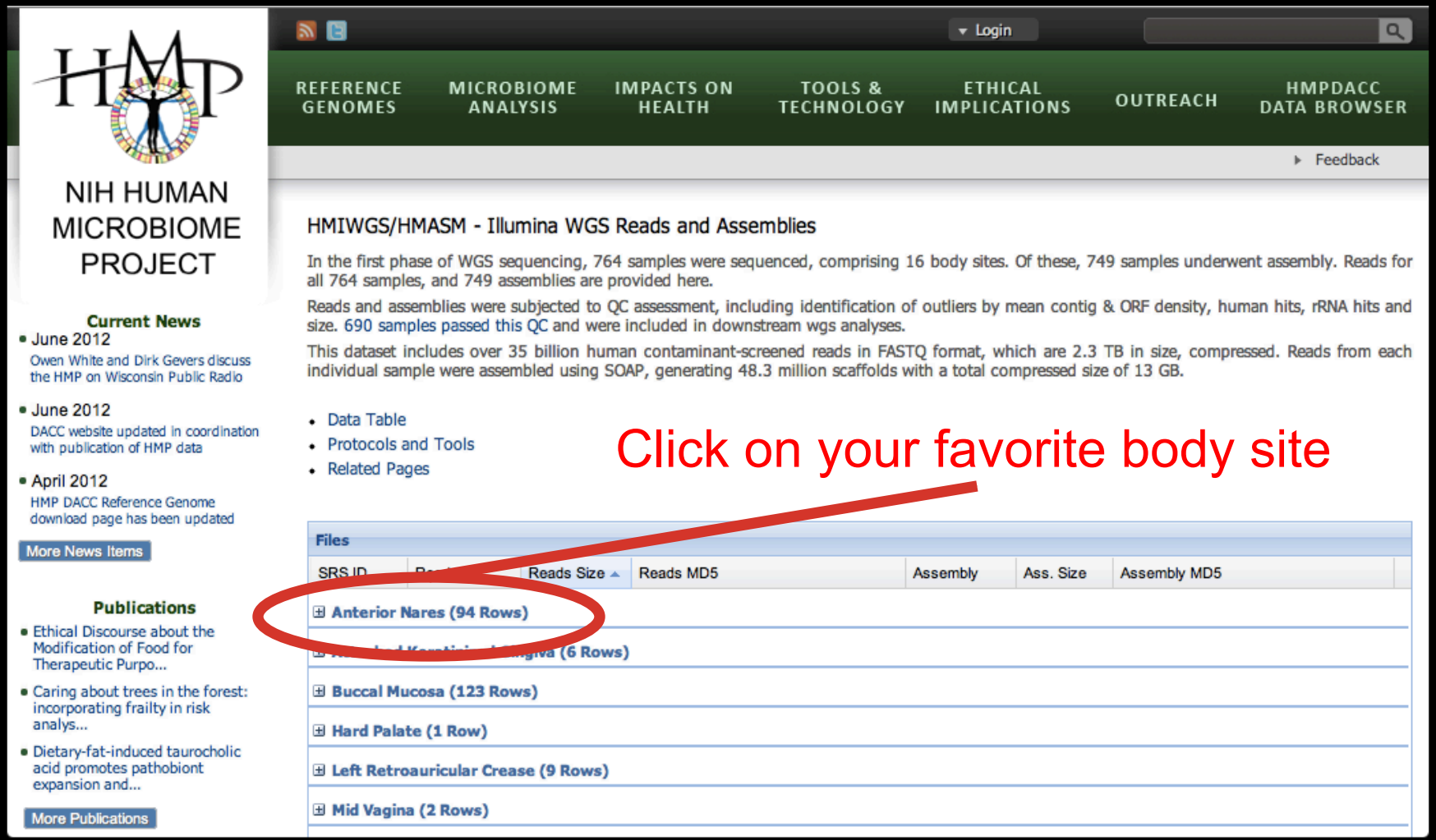
Getting some HMP data

- Check out what's available



Getting some HMP data

- Check out what's available



Getting some HMP data

Don't click on anything!

- Check out what's available

April 2012

HMP DACC Reference Genome download page has been updated

More News Items

Publications

- Ethical Discourse about the Modification of Food for Therapeutic Purpo...
- Caring about trees in the forest: incorporating frailty in risk analys...
- Dietary-fat-induced taurocholic acid promotes pathobiont expansion and...

More Publications

Data Resources

- Tools & Protocols
- BLAST against Reference Genomes
- Project Catalog
- Access to Strains
- Clinical Sampling
- Most Wanted Resource

Files

SRS ID	Reads	Reads Size	Reads MD5	Assembly	Ass. Size	Assembly MD5
Anterior Nares (94 Rows)						
SRS047708	FTP	1.7 MB	d786590ff7fec20e8967127991766029	FTP	1.3 KB	ed98eda02d80a137c52b6fa8a3c57833
SRS019215	FTP	10.1 MB	55de248bbfa8c1bbf4447d007330f7ff	FTP	12.1 KB	cab8918433280eafc3d8f6ad78dc1ff7
SRS063178	FTP	13.1 MB	336f0b31b92880224c91ad52c4784adc	FTP	10.7 KB	99de257f1942e98bf1c052e2d046df33
SRS065179	FTP	13.3 MB	27b2c9209bc56cbe219d8c65fa32296c	FTP	54.6 KB	bb8b0d62a3c1923abfcaea01a598a60a
SRS065142	FTP	13.5 MB	3b05d6fcb205106fbd03f314e39f6d63	FTP	7.6 KB	91177065cf438056f2bfc67e99562fe4
SRS018585	FTP	16.8 MB	9d4129d2f5fdd51b9fc899bd84c47b5b	FTP	7.9 KB	aa9e9857b26b9efb4fa39bfaf101dc9d
SRS015640	FTP	17.6 MB	595baf36d8b3dcdd21149b3086ccbbee	FTP	52.4 KB	1c7a464db2fccce17c02f9600c867cb1
SRS056210	FTP	18.1 MB	9b2f74b8067e6f20551e6d3b48124c42	FTP	18.3 KB	c4abace0ec0b3e7e5ce1513cb8270e56
SRS018312	FTP	18.9 MB	2454e80d7e5216adf8d5b1850c98738c	FTP	25.4 KB	4f5f760eadd77782862669263e1b1d9d
SRS015450	FTP	18.9 MB	eefc0dcf2d52ca5251b01860d54d2bb5	FTP	107.1 KB	4e0a83868f2fb44f1788dfe1aaa5e13f
SRS049744	FTP	21.5 MB	6d9e2ffc82b08ef37551e902096e4c98	FTP	14.3 KB	da7a1cddd3c84b121ff49086432d25d3
SRS012291	FTP	21.9 MB	12775f5df6e71961f1c544e84f6c7342	FTP	8.9 KB	17b5110d391817c7ce52b7c1026df1ba
SRS051600	FTP	22.2 MB	391775b95926a221b8a3cde54a79ae22	FTP	13.9 KB	6db7007edd32b534bc918aad42d600ae
SRS019339	FTP	23.1 MB	76a621d6503d11d1a133a023dc240ae5	FTP	57.3 KB	9255d8206f10ac2611cf45270daa166c
SRS017244	FTP	23.5 MB	b7c2dec67738f317cb8826c09e1a9e39	FTP	21.3 KB	9bcf59e6b4fe15a4e8ccacb0bc824ba8
SRS018671	FTP	24.0 MB	7548b06b37038440c5420f7677ff7371	FTP	135.4 KB	4a180e3ea42a46bcea0a9441b137f243

Show All | Save As CSV File

Protocols and Tools

Getting some (prepped) HMP data

- `cd` to your favorite directory and run:

```
ln -s ~/biobakery/metaphlan2/input/7*.fasta .
```

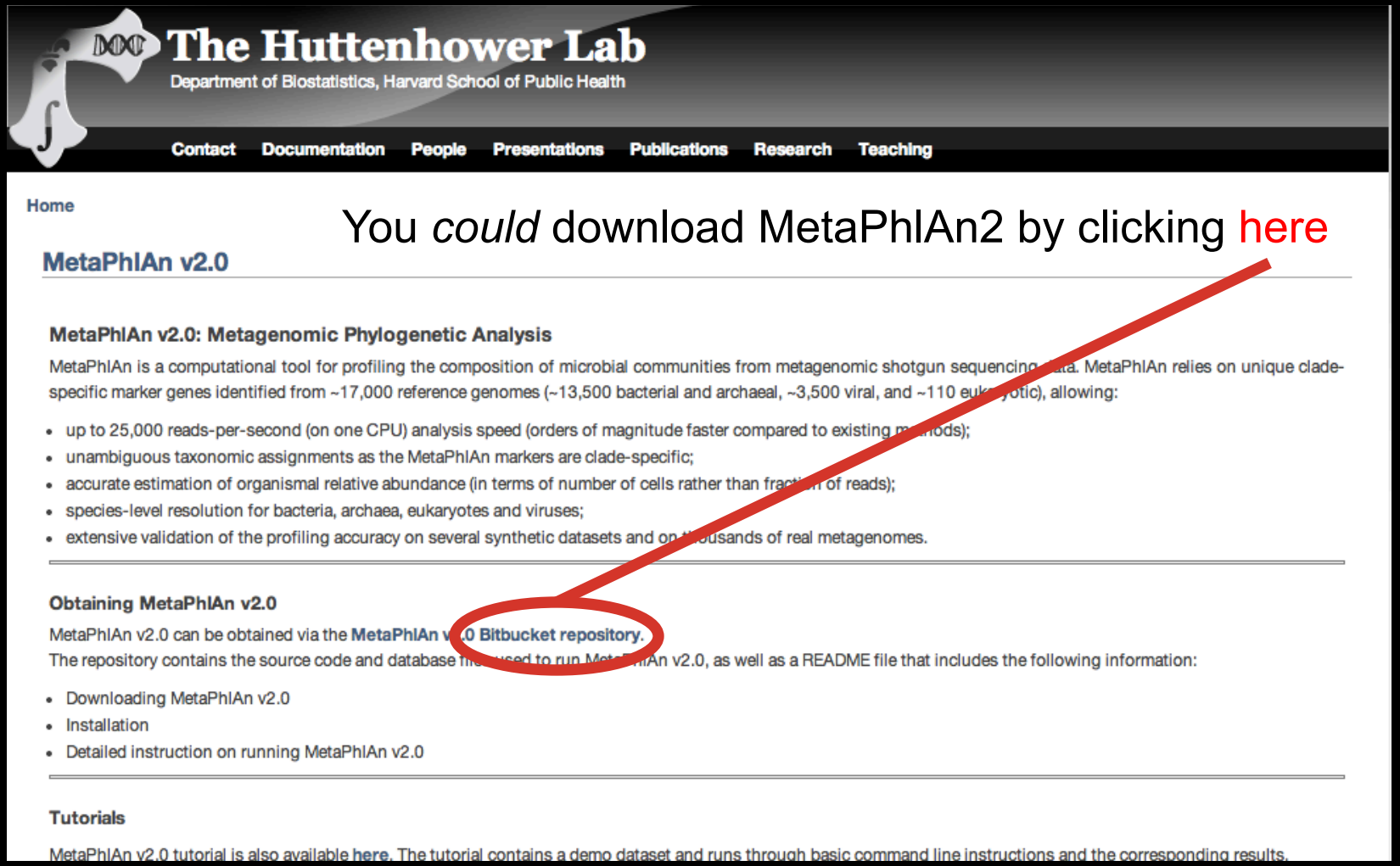
These are subsamples of six HMP files:

- SRS014459.tar.bz2 ➔ 763577454-SRS014459-Stool.fasta
- SRS014464.tar.bz2 ➔ 763577454-SRS014464-Anterior_nares.fasta
- SRS014470.tar.bz2 ➔ 763577454-SRS014470-Tongue_dorsum.fasta
- SRS014472.tar.bz2 ➔ 763577454-SRS014472-Buccal_mucosa.fasta
- SRS014476.tar.bz2 ➔ 763577454-SRS014476-Supragingival_plaque.fasta
- SRS014494.tar.bz2 ➔ 763577454-SRS014494-Posterior_fornix.fasta

- All six shotgunned body sites from
  - One subject, first visit
  - Subsampled to 20,000 reads

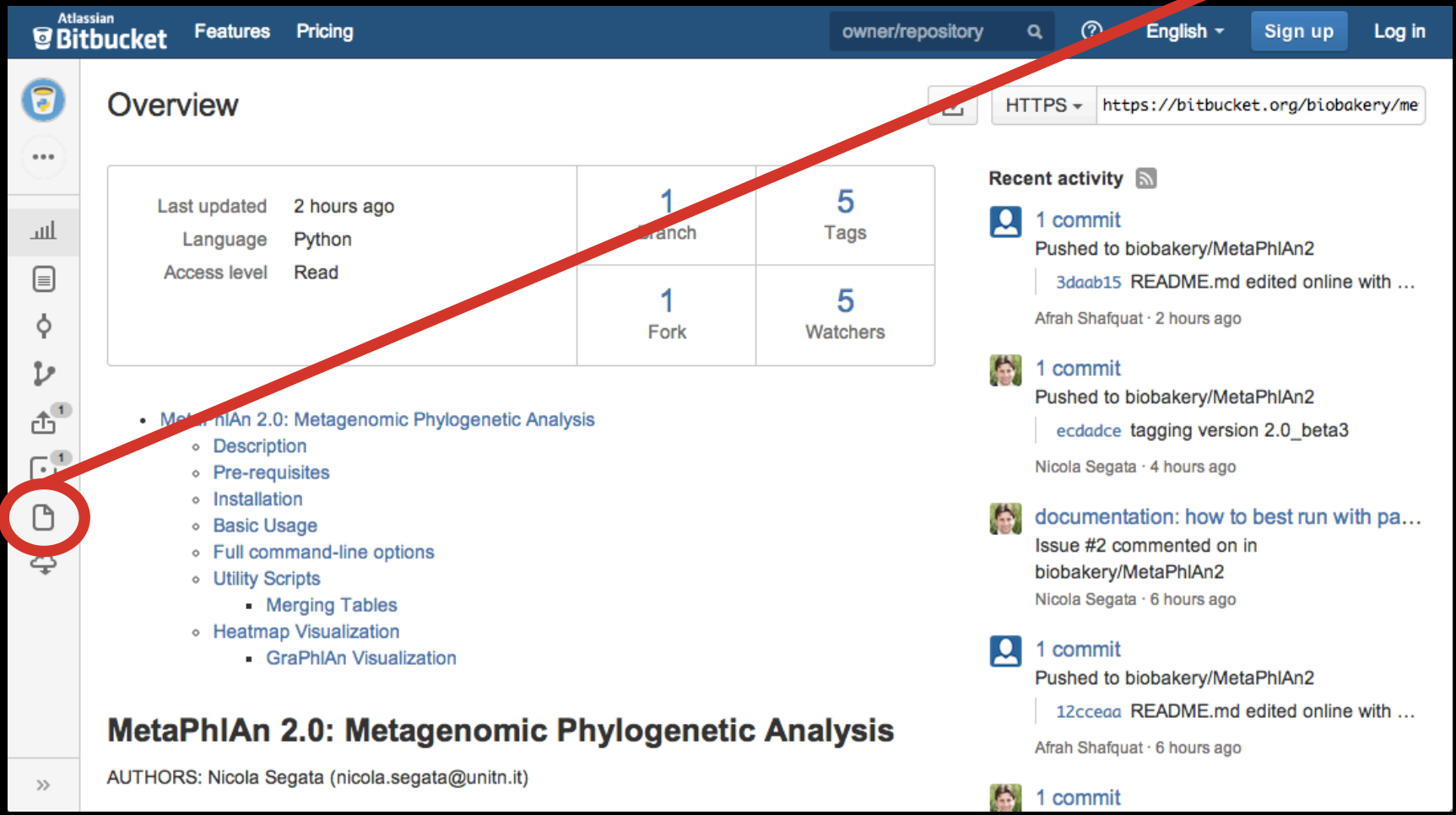
Who's there: MetaPhlAn2

- http://huttenhower.sph.harvard.edu/metaphlan2



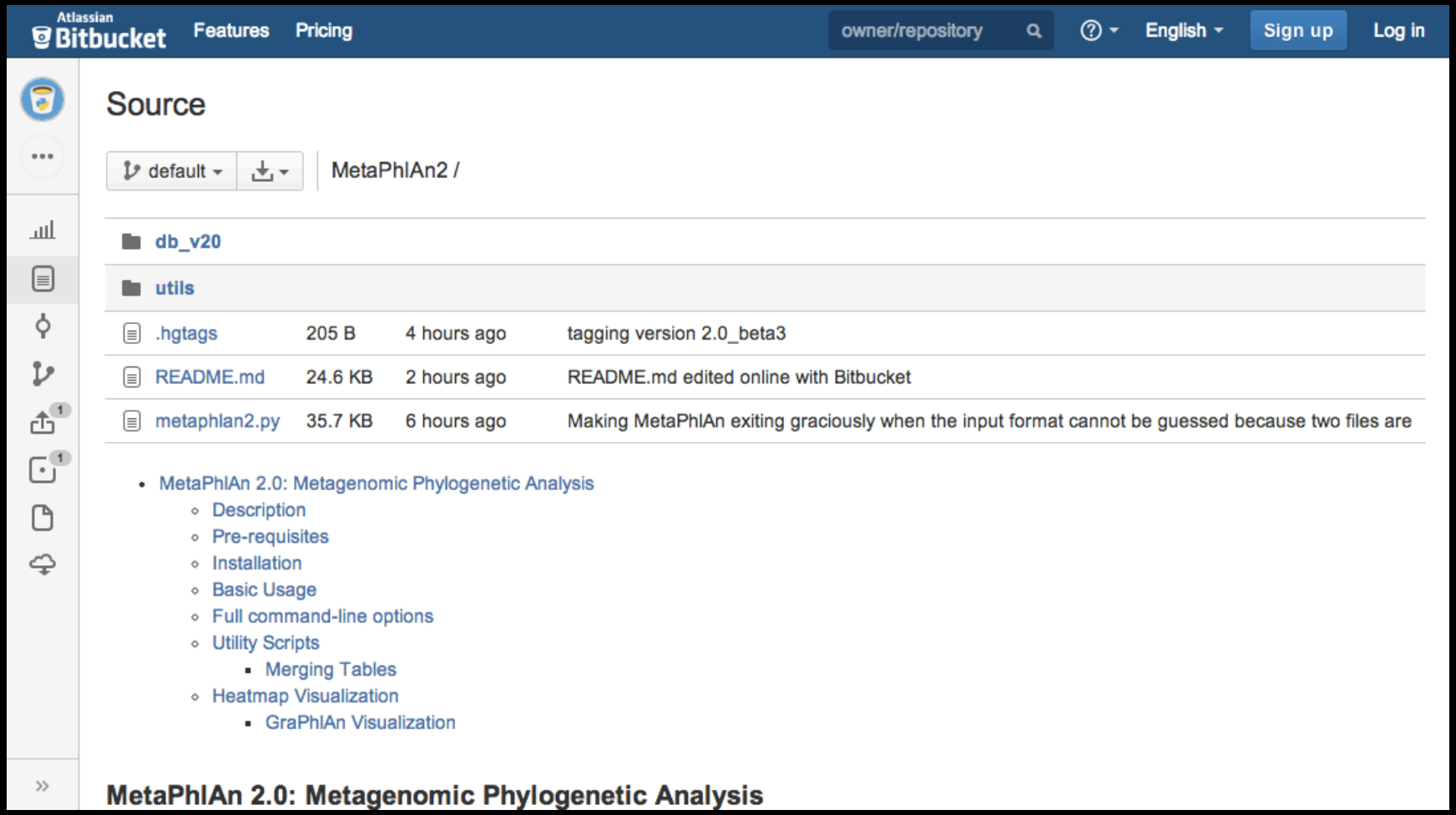
Who's there: MetaPhlAn2

- But don't! Instead, we've installed MetaPhlAn already for you by clicking here on the development site, http://bitbucket.org/biobakery/metaphlan2



Who's there: MetaPhlAn2

- The complete MetaPhlAn2 install is in `/usr/local/bioinfo/metaphlan2/`



From the command line...

- To see what you can do, run:

```
metaphlan2.py -h | less
```

  – Use the arrow keys to move up and down, `q` to quit back to the prompt

Who's there: MetaPhlAn2

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Tabs Help

ubuntu@ip-10-170-15-59: ~/galeb | ubuntu@ip-10-170-15-59: ~/galeb

```
usage: metaphlan2.py --mpa_pkl MPA_PKL --input_type
                     {fastq,fasta,multifasta,multifastq,bowtie2out,sam}
                     [--bowtie2db METAPHLAN_BOWTIE2_DB]
                     [--bt2_ps BowTie2 presets] [--bowtie2_exe BOWTIE2_EXE]
                     [--bowtie2out FILE_NAME] [--no_map] [--tmp_dir]
                     [--tax_lev TAXONOMIC_LEVEL] [--min_cu_len]
                     [--ignore_viruses] [--ignore_eukaryotes]
                     [--ignore_bacteria] [--ignore_archaea] [--stat_q]
                     [--ignore_markers IGNORE_MARKERS] [--avoid_disqm]
                     [--stat] [-t ANALYSIS TYPE] [--nreads NUMBER_OF_READS]
                     [--pres_th PRESENCE_THRESHOLD] [--clade] [--min_ab] [-h]
                     [-o output file] [--biom biom_output] [--mdelim mdelim]
                     [--nproc N] [-v]
                     [INPUT_FILE] [OUTPUT_FILE]

DESCRIPTION
 MetaPhlAn version 2.0.0 beta3 (13 August 2014):
 METAgenomic PHyLogenetic ANalysis for metagenomic taxonomic profiling.

AUTHORS: Nicola Segata (nicola.segata@unitn.it)

COMMON COMMANDS

 We assume here that metaphlan2.py is in the system path and that mpa_dir bash variable contains the
 main MetaPhlAn folder. Also BowTie2 should be in the system path with execution and read
 permissions, and Perl should be installed)

========== MetaPhlAn 2 clade-abundance estimation =================

The basic usage of MetaPhlAn 2 consists in the identification of the clades (from phyla to species a
nd
strains in particular cases) present in the metagenome obtained from a microbiome sample and their
relative abundance. This correspond to the default analysis type (--analysis_type rel_ab).
:
```

Who's there: MetaPhlAn2

- To launch your first analysis, run:

```
ln -s /usr/local/bioinf/metaphlan2/db_v20 db_v20

metaphlan2.py 763577454-SRS014459-Stool.fasta --mpa_pkl
db_v20/mpa_v20_m200.pkl --bowtie2db db_v20/mpa_v20_m200
--input_type fasta -o 763577454-SRS014459-Stool.txt
```

  - This will run for ~3-4 minutes
- What did you just do?
  - Two new output files:
    - `763577454-SRS014459-Stool.fasta.bowtie2out.txt`
      - Contains a mapping of reads to MetaPhlAn markers
    - `763577454-SRS014459-Stool.txt`
      - Contains taxonomic abundances as percentages

Who's there: MetaPhlAn

```
less 763577454-SRS014459-Stool.fasta.bowtie2out.txt
```

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Tabs Help

ubuntu@ip-10-170-15-59: ~/galeb | ubuntu@ip-10-170-15-59: ~/galeb

```
HWUSI-EAS1625_615HE:4:100:0:1248/1      gi|479140210|ref|NC_021010.1|:1043207-1044529
HWUSI-EAS1625_615HE:4:100:0:1301/1      gi|483877978|ref|NZ_KB890364.1|:31018-31902
HWUSI-EAS1625_615HE:4:100:1000:167/1    gi|242362078|ref|NZ_GG692716.1|:28261-29169
HWUSI-EAS1625_615HE:4:100:1001:1264/1   gi|270295698|ref|NZ_GG730107.1|:470181-472532
HWUSI-EAS1625_615HE:4:100:1001:1320/1   gi|224993849|ref|NZ_ACFY01000158.1|:c1296-10
HWUSI-EAS1625_615HE:4:100:1001:1604/1   gi|319644663|ref|NZ_GL635657.1|:c320982-320029
HWUSI-EAS1625_615HE:4:100:1001:1734/1   gi|484001485|ref|NZ_KB894131.1|:91019-91717
HWUSI-EAS1625_615HE:4:100:1001:259/1    gi|479210985|ref|NC_021043.1|:c1165057-1164158
HWUSI-EAS1625_615HE:4:100:1002:1501/1   gi|224485637|ref|NZ_EQ973491.1|:c620672-618312
HWUSI-EAS1625_615HE:4:100:1003:1644/1   gi|224485636|ref|NZ_EQ973490.1|:c204903-202990
HWUSI-EAS1625_615HE:4:100:1003:1702/1   gi|423335209|ref|NZ_JH976498.1|:329186-330046
HWUSI-EAS1625_615HE:4:100:1003:2030/1   gi|238922432|ref|NC_012781.1|:2910912-2912072
HWUSI-EAS1625_615HE:4:100:1004:353/1    gi|223955873|ref|NZ_DS499674.1|:c266282-265248
HWUSI-EAS1625_615HE:4:100:1004:742/1    gi|283767237|ref|NZ_GG730311.1|:c124395-124171
HWUSI-EAS1625_615HE:4:100:1005:1722/1   gi|410105720|ref|NZ_JH976502.1|:750498-751148
HWUSI-EAS1625_615HE:4:100:1005:505/1    gi|479170689|ref|NC_021020.1|:1540599-1542305
HWUSI-EAS1625_615HE:4:100:1006:848/1    gi|347530298|ref|NC_015977.1|:c3433030-3431387
HWUSI-EAS1625_615HE:4:100:1007:1428/1   gi|423332908|ref|NZ_JH976496.1|:1485161-1487113
HWUSI-EAS1625_615HE:4:100:1007:1465/1   gi|423332908|ref|NZ_JH976496.1|:906255-909584
HWUSI-EAS1625_615HE:4:100:1008:1187/1   gi|224485479|ref|NZ_EQ973214.1|:108053-108250
HWUSI-EAS1625_615HE:4:100:1008:1241/1   gi|270293478|ref|NZ_GG730105.1|:c830784-828727
HWUSI-EAS1625_615HE:4:100:1008:140/1    gi|224514921|ref|NZ_DS499545.1|:41991-42827
HWUSI-EAS1625_615HE:4:100:1009:154/1    gi|301307949|ref|NZ_GG774972.1|:644845-649113
HWUSI-EAS1625_615HE:4:100:1009:467/1    gi|303257489|ref|NZ_GL383997.1|:67163-67873
HWUSI-EAS1625_615HE:4:100:1009:596/1    gi|423290212|ref|NZ_JH724228.1|:c907457-905856
HWUSI-EAS1625_615HE:4:100:1009:82/1     gi|479213596|ref|NC_021044.1|:c1569840-1568455
HWUSI-EAS1625_615HE:4:100:100:193/1     gi|224514888|ref|NZ_DS499516.1|:148626-150644
HWUSI-EAS1625_615HE:4:100:100:866/1     gi|345651619|ref|NZ_JH114362.1|:c62469-60163
HWUSI-EAS1625_615HE:4:100:1010:1731/1   gi|479213596|ref|NC_021044.1|:1831714-1832487
763577454-SRS014459-Stool.bowtie2out.txt
```

Who's there: MetaPhlAn

```
less 763577454-SRS014459-Stool.txt
```

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Tabs Help

ubuntu@ip-10-170-15-59: ~/galeb | ubuntu@ip-10-170-15-59: ~/galeb

```
k__Bacteria     100.0
k__Bacteria|p__Firmicutes       64.82041
k__Bacteria|p__Bacteroidetes    35.17959
k__Bacteria|p__Firmicutes|c__Clostridia 64.82041
k__Bacteria|p__Bacteroidetes|c__Bacteroidia     35.17959
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales        64.82041
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales    35.17959
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae     37.71449
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae  31.50008
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Eubacteriaceae      21.99035
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae     5.11557
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Porphyromonadaceae      3.67952
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae|g__Subdoligranulum  37.7
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides   31.5
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Eubacteriaceae|g__Eubacterium       21.9
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Roseburia        5.11
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Porphyromonadaceae|g__Parabacteroide
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae|g__Subdoligranulum|s__Su
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Eubacteriaceae|g__Eubacterium|s__Eubacte
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bac
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bac
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Eubacteriaceae|g__Eubacterium|s__Eubacte
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Roseburia|s__Roseburi
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bac
:
```

Who's there: MetaPhlAn2

- Now do the Anterior_nares sample:

```
$ metaphlan2.py 763577454-SRS014459-Stool.fasta --mpa_pkl
db_v20/mpa_v20_m200.pkl --bowtie2db db_v20/mpa_v20_m200
--input_type fasta -o 763577454-SRS014459-Stool.txt
...
```

  - Note that you can use the up arrow key to make your life easier!
- Usually, you would write a script to analyze all the samples…

Who's there: MetaPhlAn2

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Tabs Help

ubuntu@ip-10-170-15-59: ~/galeb

```
#!/usr/bin/env bash

for file in *.fasta
do
    echo Now analyzing file $file
    name=`expr "$file" : '\(.*\).fasta'`
    metaphlan2.py $file \
        --mpa_pkl db_v20/mpa_v20_m200.pkl \
        --bowtie2db db_v20/mpa_v20_m200 \
        --input_type fasta \
        -o $name".txt"
done
```

But let's copy the rest pre-calculated ☺

```
cp ~/biobakery/metaphlan2/output/*.txt .
```

Who's there: MetaPhlAn2

- Let's make a single table containing all six samples:

```
mkdir tmp

mv *.bowtie2out.txt tmp

~/biobakery/metaphlan2/utils/
   merge_metaphlan_tables.py *.txt > 763577454.tsv
```

- You can look at this file using `less`
  - Note 1: The arguments `less -x4 -S` will help
  - Note 2: You can set this "permanently" using `export LESS="-x4 -S"`

Who's there: MetaPhlAn2

```
less -x4 -S 763577454.tsv
```

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Tabs Help

ubuntu@ip-10-170-15-59: ~/galeb | ubuntu@ip-10-170-15-59: ~/galeb

```
ID  763577454-SRS014459-Stool   763577454-SRS014464-Anterior_nares  763577454-SRS014470-Tongue_dorsum   763577454-SRS014472-Buccal_mucosa
k__Bacteria 100.0   16.77458    100.0   100.0   100.0   100.0
k__Bacteria|p__Actinobacteria   0.0 14.03084    0.86835 0.0 90.34242    0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria 0.0 14.03084    0.86835 0.0 90.34242    0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales  0.0 14.03084    0.86835 0.0 90.34242    0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Actinomycetaceae  0.0 0.0 0.86835 0.0 0.0 0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Actinomycetaceae|g__Actinomyces   0.0 0.0 0.86835 0.0 0.0 0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Actinomycetaceae|g__Actinomyces|s__Actinomyces_graevenitzii   0.0 0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Actinomycetaceae|g__Actinomyces|s__Actinomyces_graevenitzii|t__Actino
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Corynebacteriaceae    0.0 14.03084    0.0 0.0 58.1475 0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Corynebacteriaceae|g__Corynebacterium 0.0 14.03084    0.0 0.0 58.1475
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Corynebacteriaceae|g__Corynebacterium|s__Corynebacterium_matruchotii
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Corynebacteriaceae|g__Corynebacterium|s__Corynebacterium_matruchotii|
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Corynebacteriaceae|g__Corynebacterium|s__Corynebacterium_pseudodiphth
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Corynebacteriaceae|g__Corynebacterium|s__Corynebacterium_pseudodiphth
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Micrococcaceae    0.0 0.0 0.0 0.0 32.19492    0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Micrococcaceae|g__Rothia  0.0 0.0 0.0 0.0 32.19492    0.0
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Micrococcaceae|g__Rothia|s__Rothia_dentocariosa   0.0 0.0 0.0 0.0 32.
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Micrococcaceae|g__Rothia|s__Rothia_dentocariosa|t__Rothia_dentocarios
k__Bacteria|p__Bacteroidetes    35.17959    0.0 24.49606    0.0 9.65758 0.0
k__Bacteria|p__Bacteroidetes|c__Bacteroidia 35.17959    0.0 24.49606    0.0 0.0 0.0
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales    35.17959    0.0 24.49606    0.0 0.0 0.0
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae  31.50008    0.0 0.0 0.0 0.0 0.0
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides   31.50008    0.0 0.0 0.0 0.0 0.0
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bacteroides_cellulosilyticus   3.82377 0.0
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bacteroides_cellulosilyticus|t__Bacteroide
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bacteroides_massiliensis   10.67098    0.0
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bacteroides_massiliensis|t__Bacteroides_ma
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacteroides|s__Bacteroides_ovatus 4.08388 0.0 0.0 0.0 0.0
763577454.tsv
```

Who's there: MetaPhlAn2

```
sed "s/.*|//" 763577454.tsv | less -x4 -S
```

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Tabs Help

ubuntu@ip-10-170-15-59: ~/galeb | ubuntu@ip-10-170-15-59: ~/galeb

```
ID  763577454-SRS014459-Stool   763577454-SRS014464-Anterior_nares  763577454-SRS014470-Tongue_dorsum   763577454-SRS014472-Buccal_mucosa
k__Bacteria 100.0   16.77458    100.0   100.0   100.0   100.0
p__Actinobacteria   0.0 14.03084    0.86835 0.0 90.34242    0.0
c__Actinobacteria   0.0 14.03084    0.86835 0.0 90.34242    0.0
o__Actinomycetales  0.0 14.03084    0.86835 0.0 90.34242    0.0
f__Actinomycetaceae 0.0 0.0 0.86835 0.0 0.0 0.0
g__Actinomyces  0.0 0.0 0.86835 0.0 0.0 0.0
s__Actinomyces_graevenitzii 0.0 0.0 0.86835 0.0 0.0 0.0
t__Actinomyces_graevenitzii_unclassified    0.0 0.0 0.86835 0.0 0.0 0.0
f__Corynebacteriaceae   0.0 14.03084    0.0 0.0 58.1475 0.0
g__Corynebacterium  0.0 14.03084    0.0 0.0 58.1475 0.0
s__Corynebacterium_matruchotii  0.0 0.0 0.0 0.0 58.1475 0.0
t__Corynebacterium_matruchotii_unclassified 0.0 0.0 0.0 0.0 58.1475 0.0
s__Corynebacterium_pseudodiphtheriticum 0.0 14.03084    0.0 0.0 0.0 0.0
t__GCF_000466825    0.0 14.03084    0.0 0.0 0.0 0.0
f__Micrococcaceae   0.0 0.0 0.0 0.0 32.19492    0.0
g__Rothia   0.0 0.0 0.0 0.0 32.19492    0.0
s__Rothia_dentocariosa  0.0 0.0 0.0 0.0 32.19492    0.0
t__Rothia_dentocariosa_unclassified 0.0 0.0 0.0 0.0 32.19492    0.0
p__Bacteroidetes    35.17959    0.0 24.49606    0.0 9.65758 0.0
c__Bacteroidia  35.17959    0.0 24.49606    0.0 0.0 0.0
o__Bacteroidales    35.17959    0.0 24.49606    0.0 0.0 0.0
f__Bacteroidaceae   31.50008    0.0 0.0 0.0 0.0 0.0
g__Bacteroides  31.50008    0.0 0.0 0.0 0.0 0.0
s__Bacteroides_cellulosilyticus 3.82377 0.0 0.0 0.0 0.0 0.0
t__Bacteroides_cellulosilyticus_unclassified    3.82377 0.0 0.0 0.0 0.0 0.0
s__Bacteroides_massiliensis 10.67098    0.0 0.0 0.0 0.0 0.0
t__Bacteroides_massiliensis_unclassified    10.67098    0.0 0.0 0.0 0.0 0.0
s__Bacteroides_ovatus   4.08388 0.0 0.0 0.0 0.0 0.0
:
```

Who's there: MetaPhlAn2

```
$ sed "s/.*|//" 763577454.tsv | sort -k 3 -n -r
| column -t | less -x4 -S
```

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Tabs Help

ubuntu@ip-10-170-15-59: ~/galeb | ubuntu@ip-10-170-15-59: ~/galeb

ID	763577454-SRS014459-Stool	763577454-SRS014464-Anterior_nares	763577454-SRS014470-T
t__PRJNA66339	0.0	83.22542	0.0
s__Propionibacterium_phage_PAS50	0.0	83.22542	0.0
p__Viruses_noname	0.0	83.22542	0.0
o__Caudovirales	0.0	83.22542	0.0
k__Viruses	0.0	83.22542	0.0
g__Siphoviridae_noname	0.0	83.22542	0.0
f__Siphoviridae	0.0	83.22542	0.0
c__Viruses_noname	0.0	83.22542	0.0
k__Bacteria	100.0	16.77458	100.0
t__GCF_000466825	0.0	14.03084	0.0
s__Corynebacterium_pseudodiphtheriticum	0.0	14.03084	0.0
p__Actinobacteria	0.0	14.03084	0.86835
o__Actinomycetales	0.0	14.03084	0.86835
g__Corynebacterium	0.0	14.03084	0.0
f__Corynebacteriaceae	0.0	14.03084	0.0
c__Actinobacteria	0.0	14.03084	0.86835
t__GCF_000245815	0.0	2.74374	0.0
s__Dolosigranulum_pigrum	0.0	2.74374	0.0
p__Firmicutes	64.82041	2.74374	74.63559
o__Lactobacillales	0.0	2.74374	24.37049
g__Dolosigranulum	0.0	2.74374	0.0
f__Carnobacteriaceae	0.0	2.74374	0.0
c__Bacilli	0.0	2.74374	24.37049
t__Veillonella_atypica_unclassified	0.0	0.0	16.35219
t__Streptococcus_salivarius_unclassified	0.0	0.0	3.16128
t__Streptococcus_parasanguinis_unclassified	0.0	0.0	21.20921
t__Streptococcus_mitis_oralis_pneumoniae_unclassified	0.0	0.0	0.0
t__Rothia_dentocariosa_unclassified	0.0	0.0	0.0

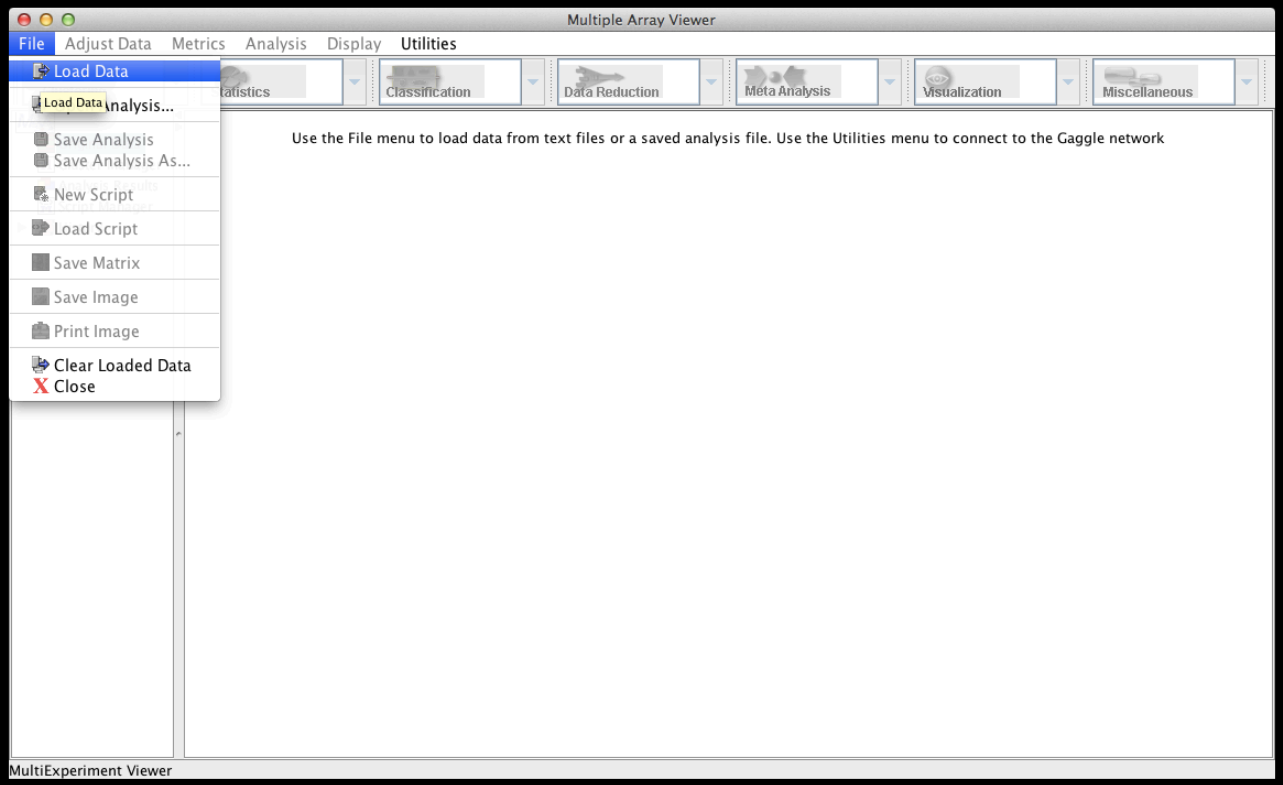
:

Who's there: MetaPhlAn

- But it's easier using MeV; http://www.tm4.org/mev.html
- `cd` to /home/ubuntu/biobakery/mev
- Double click tmev.sh > Run

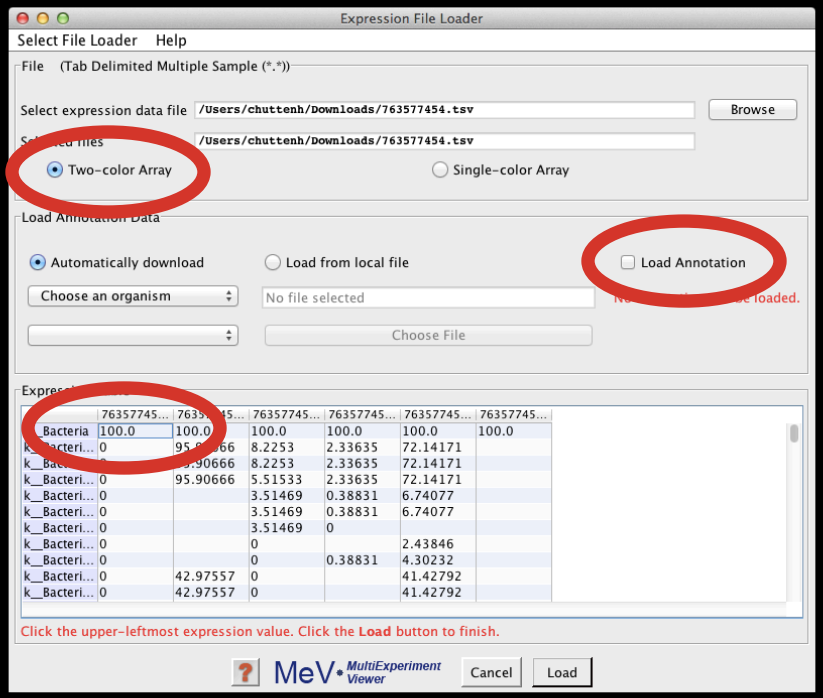
An interlude: MeV

- Launch MeV, select File/Load data



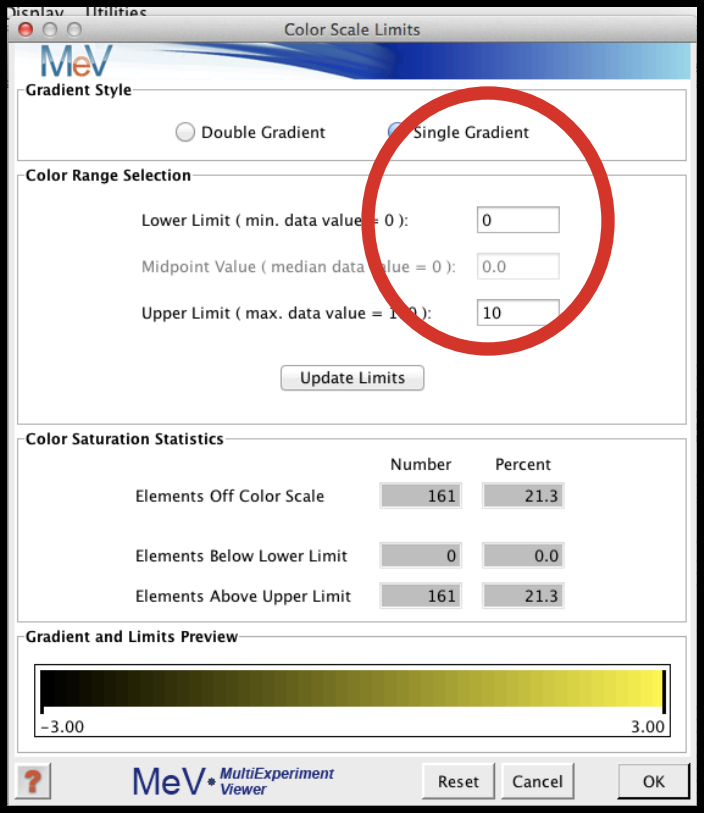
An interlude: MeV

- Click “Browse” to your TSV file, then
  - Tell MeV it’s a two-color array
  - Uncheck “Load annotation”
  - Click on the upper-leftmost ***data*** value



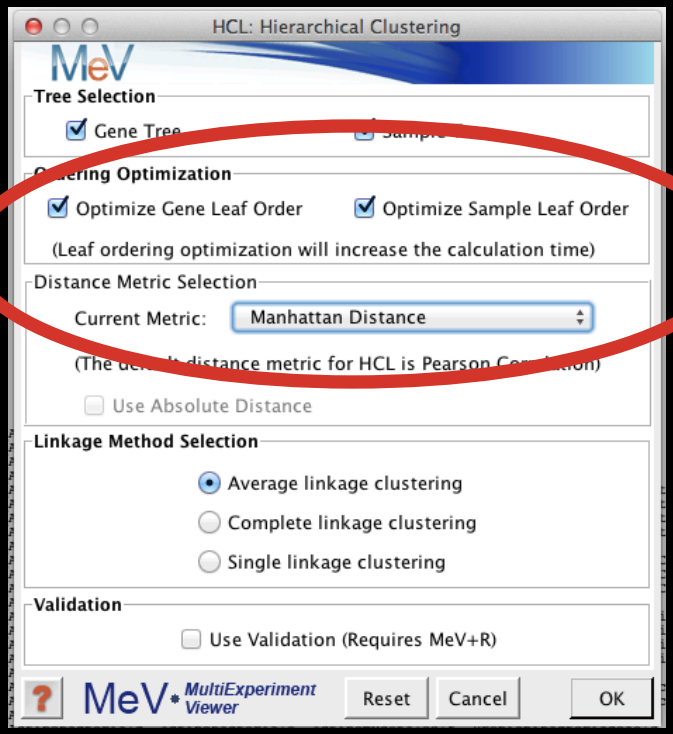
An interlude: MeV

- "Load" your data, then make it visible by:
  - Display/Set Color Scale Limits
  - Choose Single Gradient, min 0, max 10



An interlude: MeV

- Finally, to play around a bit:
  - Display/Set Element Size/whatever you'd like
  - Clustering/Hierarchical Clustering
  - Optimize both gene and sample order
  - And select Manhattan Distance (imperfect!)



An interlude: MeV

- If you'd like, you can
  - Display/Sample-Column Labels/Abbr. Names

An interlude: MeV

- MeV is a tool; imperfect, but convenient
  - You should likely include just “leaf” nodes
    - Species, whose names start include “s__”
    - You can filter your file using:

```
cat 763577454.tsv | grep -E '(Stool)|(s__)' >
763577454_species.tsv
```

  - You can, but might not want to, z-score normalize
    - Adjust Data/Gene-Row Adjustments/Normalize Genes-Rows

- Many other tools built in – experiment!

The two big questions…

Who is there?

What are they doing?

Sample #	1	2	3	4	5	6
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
Clade1|Bug1	0.40	0.56	0.07	0.31	0.42	0.27
Clade1|Bug2	0.00	0.30	0.36	0.37	0.04	0.05
Clade2	0.60	0.13	0.57	0.32	0.53	0.68
Clade2|Bug3	0.11	0.00	0.10	0.32	0.15	0.23
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

The ~~two~~ three big questions…

Who is there?

What are they doing?

What does it all mean?

Sample #	1	2	3	4	5	6
Profession	Student	Postdoc	Postdoc	Professor	Student	Student
Gender	Male	Female	Female	Male	Male	Female
Site	Oral	Gut	Oral	Gut	Oral	Gut
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
Clade1|Bug1	0.40	0.56	0.07	0.31	0.42	0.27
Clade1|Bug2	0.00	0.30	0.36	0.37	0.04	0.05
Clade2	0.60	0.13	0.57	0.32	0.53	0.68
Clade2|Bug3	0.11	0.00	0.10	0.32	0.15	0.23
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

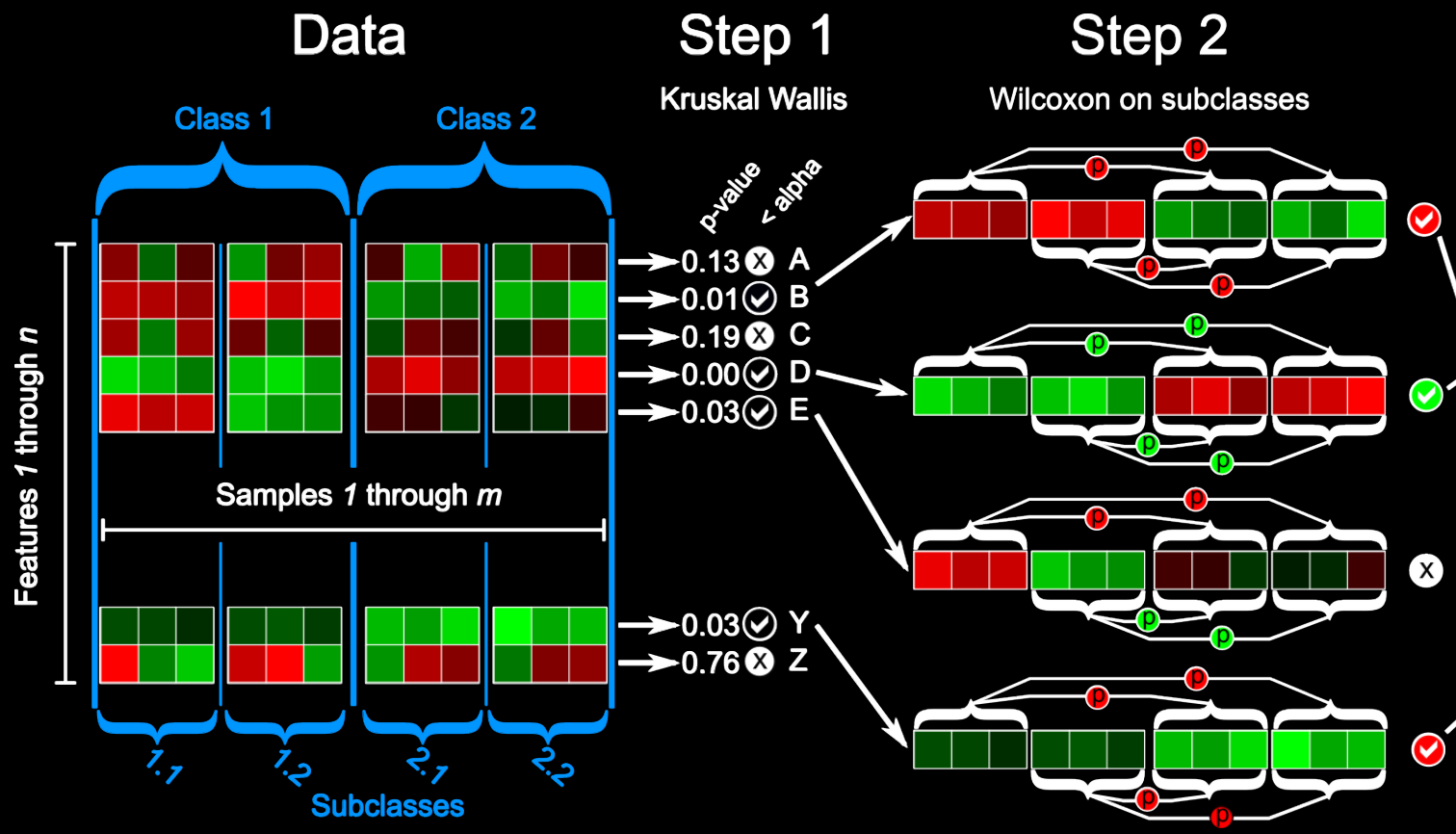
Properties of microbiome data

- General problem: correlate microbiome features with metadata (potentially controlling for other features)
- Intuitively summarize the results

Sample #	1	2	3	4	5	6
Profession	Student	Postdoc	Postdoc	Professor	Student	Student
Gender	Male	Female	Female	Male	Male	Female
Site	Oral	Gut	Oral	Gut	Oral	Gut
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
Clade1|Bug1	0.40	0.56	0.07	0.31	0.42	0.27
Clade1|Bug2	0.00	0.30	0.36	0.37	0.04	0.05
Clade2	0.60	0.13	0.57	0.32	0.53	0.68
Clade2|Bug3	0.11	0.00	0.10	0.32	0.15	0.23
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

LEfSe: LDA Effect Size
Finding metagenomic biomarkers

Nicola Segata

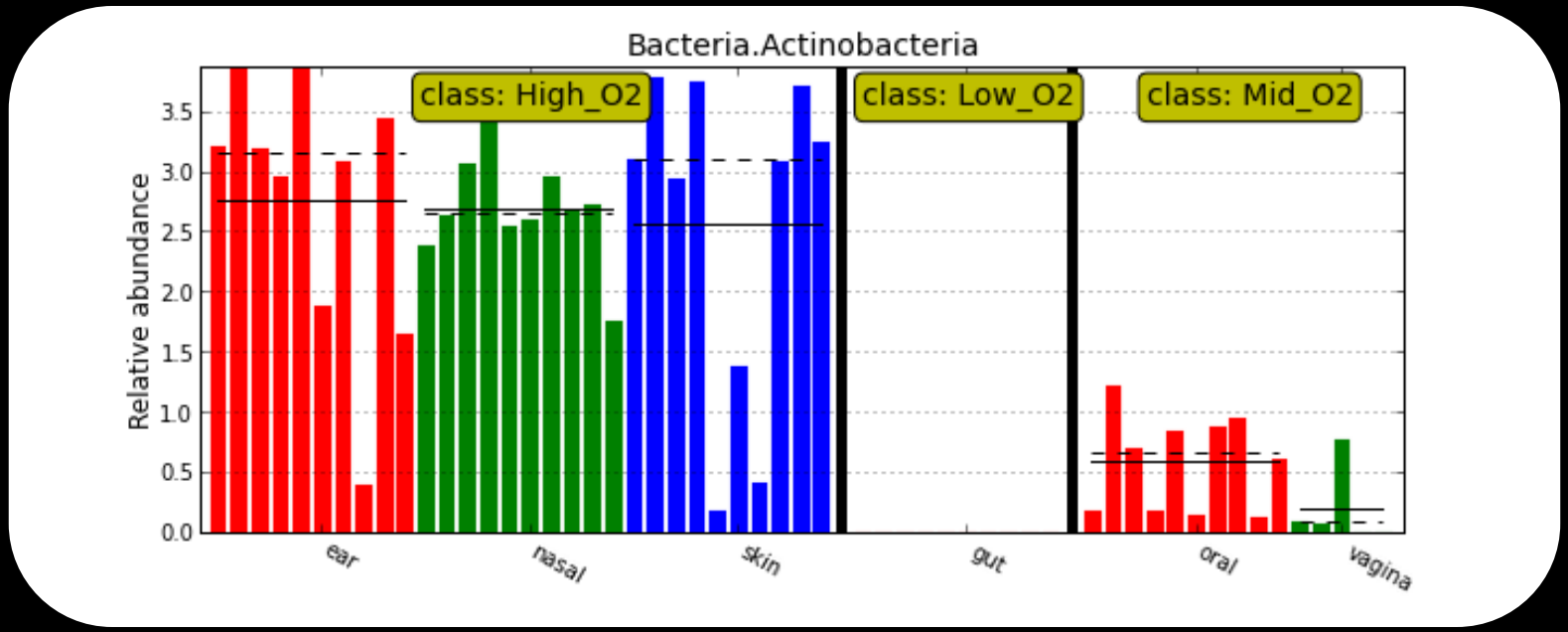


A Statistical consistency

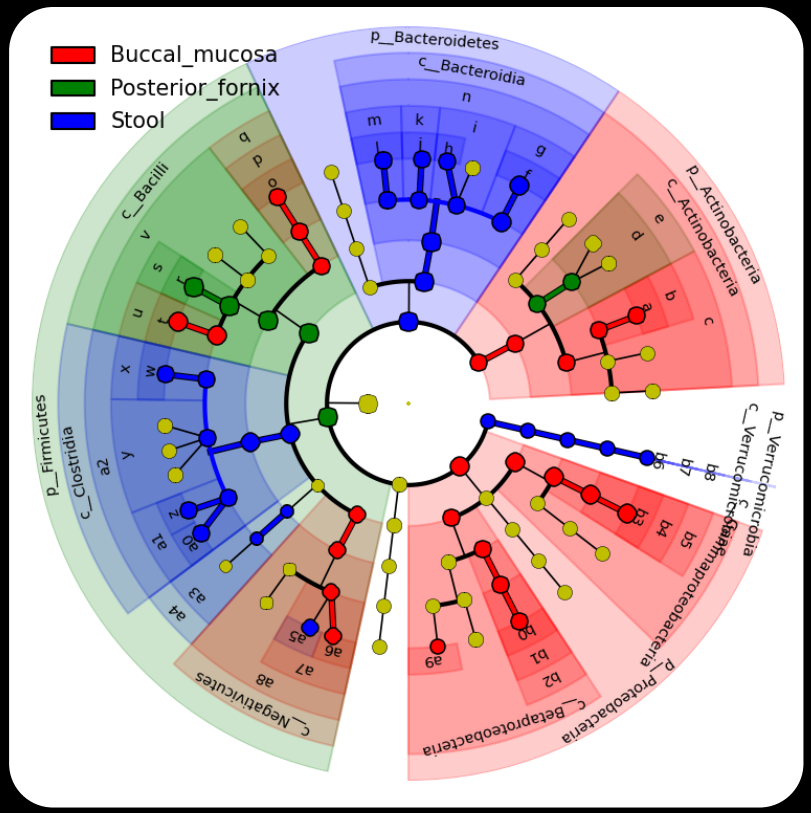
B Biological consistency

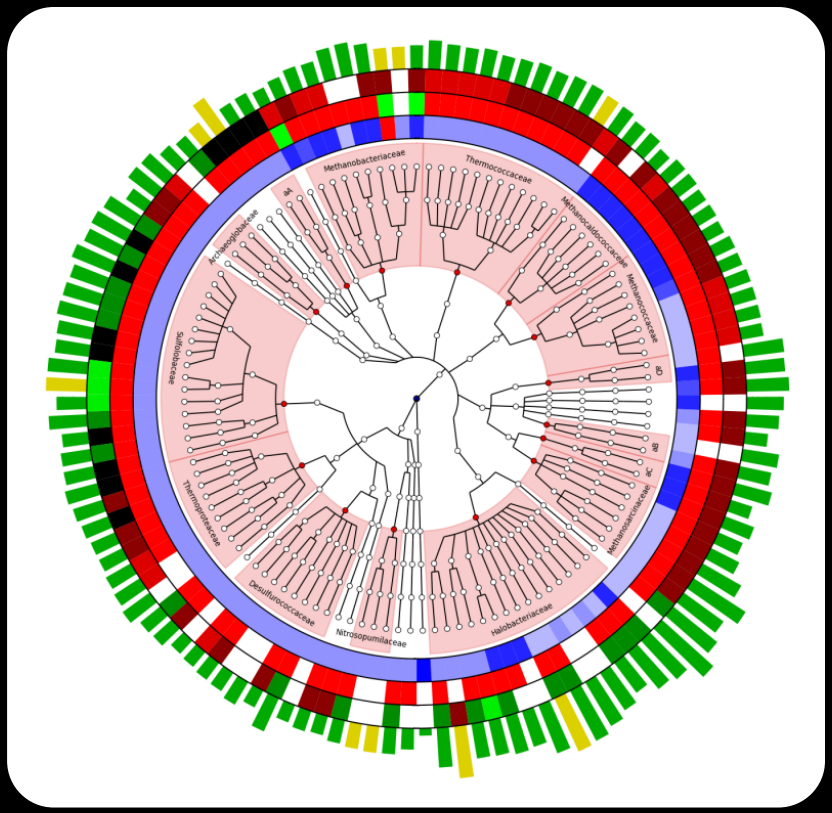
C Biological Effect size

Example LEfSe application:
Find O_2-loving bugs (controlling for body site)



Superimpose enrichments on the tree of life using GraPhlAn





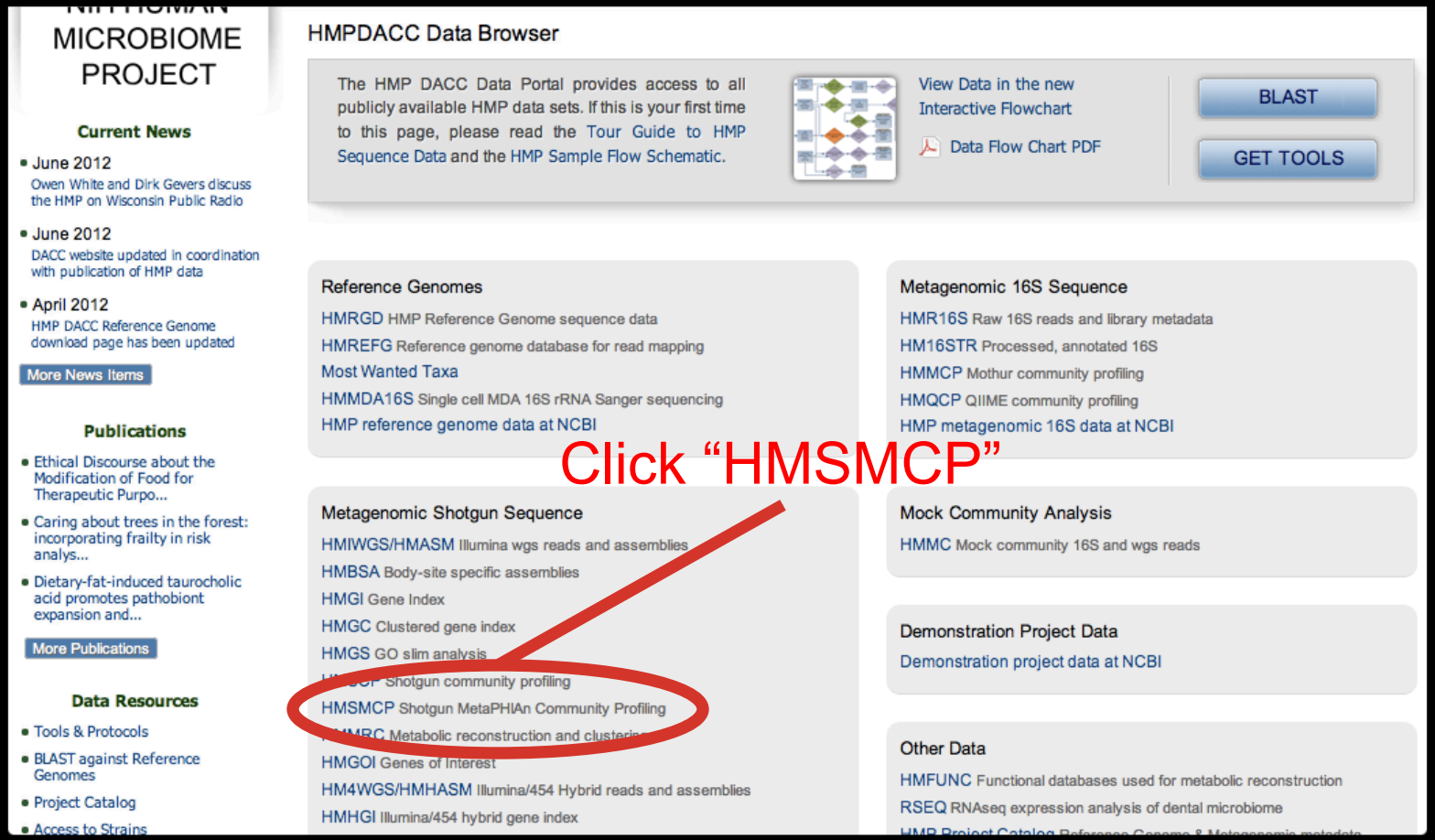
LEfSe Associations

Metadata Rings

http://huttenhower.sph.harvard.edu/graphlan

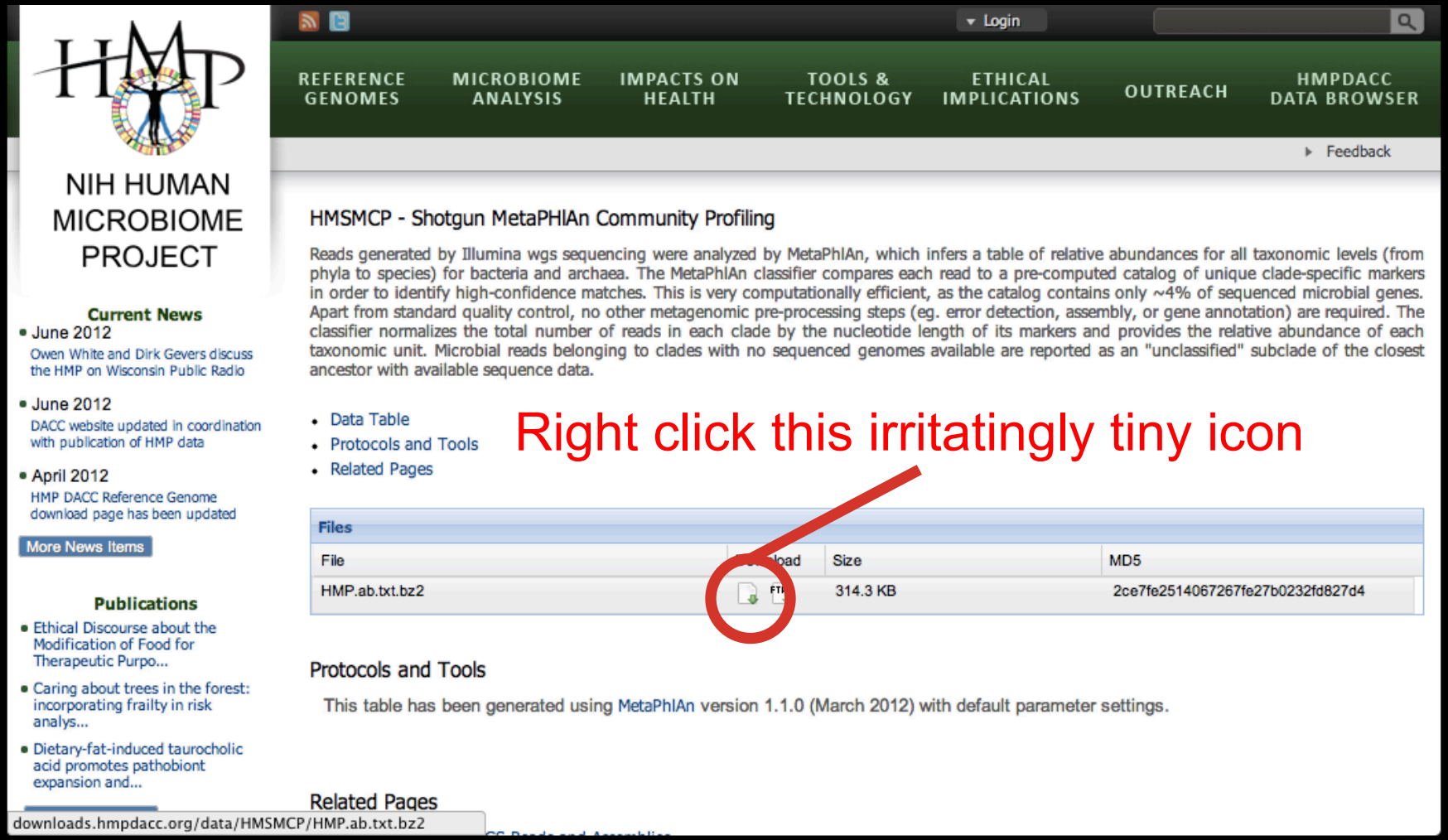
What it means: LEfSe

- Let's get *all* of the HMP species data: http://hmpdacc.org/resources/data_browser.php



What it means: LEfSe

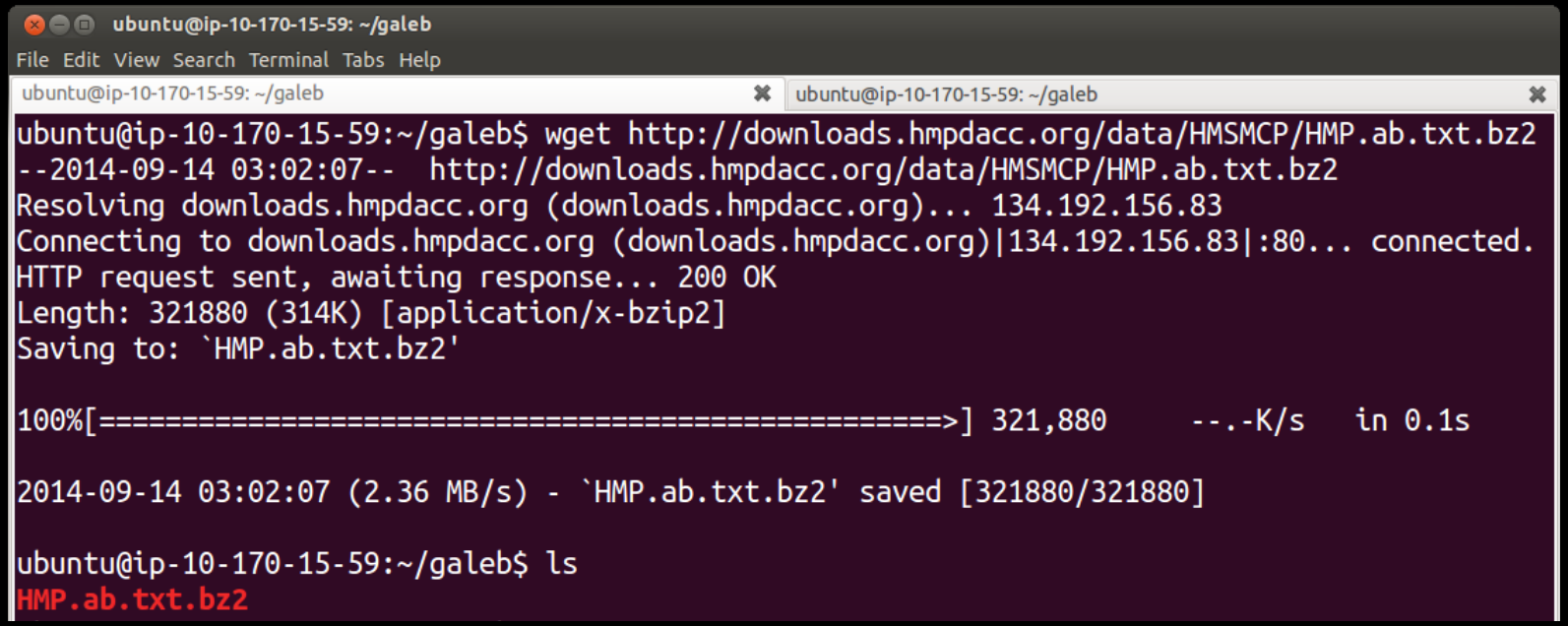
- Download the MetaPhlAn1 table for all 700 samples



Downloading from the command line

- Instead of saving this, download it by:
  - Right-click to copy the URL
  - Run

    `wget <paste URL here>`
  - Note: `curl -O <URL>` works just as well



What it means: LEfSe

- Make sure this file is in your current directory, and expand it:

```
bunzip2 HMP.ab.txt.bz2
```

- Look at the result

```
less -S HMP.ab.txt
```

- **IMPORTANT!!!**
  - This file's too big to analyze directly today

```
ln -s ~/biobakery/data/HMP.ab.filtered.txt
```

- This is great – tons of data, but no metadata
  - Scripts and data from HUMAnN to the rescue:

```
~/biobakery/metadata.py ~/biobakery/data/hmp_metadata.dat
    < HMP.ab.filtered.txt > HMP.ab.filtered.metadata.tsv
```

- NOW take a look again

What it means: LEfSe

HMP.ab.filtered.metadata.tsv - LibreOffice Calc

File Edit View Insert Format Tools Data Window Help

Liberation Sans 10

A1 sid

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	sid	SRS043001	SRS017127	SRS021473	SRS011134	SRS050184	SRS011529	SRS048164	SRS016516	SRS052330	SRS011355	SRS011452	SRS019787	SRS054776	SRS024140	SRS014683	SRS016018
2	RANDSID	550534656	159551223	158479027	158499257	508703490	159166850	861967750	159753524	765640925	158944319	159146620	764669880	764224817	159207311	763961826	76444734
3	START	Q3_2009	Q2_2009	Q1_2009	Q1_2009	Q3_2009	Q2_2009	Q3_2009	Q2_2009	Q3_2009	Q1_2009	Q1_2009	Q2_2009	Q2_2009	Q2_2009	Q1_2009	Q2_2009
4	GENDER	female	male	male	male	female	male	male	female	female	female	male	male	male	male	male	male
5	VISNO	1	1	2	1	1	1	1	1	1	1	1	2	2	2	1	
6	STSite	Stool	Buccal_mucosa	Buccal_mucosa	Stool	Posterior_fornix	Stool	Stool	Posterior_fornix	Posterior_fornix	Posterior_fornix	Stool	Stool	Buccal_mucosa	Buccal_mucosa	Stool	Stool
7	Parent_Specimen	700106291	700033688	700097185	700014832	700038759	700016608	700038870	700032243	700038805	700015577	700016136	700038231	700106652	700100608	700023337	70002464
8	Run ID	704GE	61HJLAAXX	61K2LAAXX	61JGUAAXX	704N4	61PNF	61NTL	61VKUAAXX	7055M	61KYVAAXX	61WER	704MU	621F6	61JD1AAXX	614NM	61PPR
9	Lane	6	6	7	6	8	5	1	4	1	8	2	4	5	2	1	
10	SRS	700106291	700033689	700097185	700014837	700038759	700016610	700038870	700032243	700038805	700015579	700016142	700038263	700106652	700100608	700023337	70002467
11	Mean Quality	29.75	29	33	27	31.07	31.92	33.16	33	32.91	24	32.97		32.65	29		34.2
12	Number of Quality Bases	5938136715	6349906779	4784765427	6210952530	5330742538	6605008593	4654538314	5233442453	5439436415	3621291754	2749895648		5709503626	6823557644		393099251
13	Percent of Human Reads	0.0024	0.6746	0.8842	0.0002	0.7872	0.0004	0.0002	0.8342	0.7861	0.8857	0.0043		0.734	0.865		0.000
14	Unique Non-Human Bases	6779445369	2611543625	959383209	7794356797	1264901720	7187185125	4912150763	5230564060	1234292763	683832927	2252544600		1607164515	1336140118		410671756
15	k__Bacteria|p__Proteobact	0	0	0	0.59019	0	0.15046	1.46625	0	0	0	0	0	0	0	0	
16	k__Bacteria|p__Actinobact	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	k__Bacteria|p__Bacteroide	2.57633	0	0	4.20761	0	5.52547	8.52942	0	0	0	15.90901	1.27072	0.14801	1.19015	6.11771	11.5346
18	k__Bacteria|p__Firmicutes	0.09916	0	0.00175	0.33665	0	0.10695	0.93543	0	0	0	1.85125	0.07391	0	0.35352	0.14181	1.3823
19	k__Bacteria|p__Firmicutes	0	0	0	18.31739	0	0	0	0	0	0	0	0	0	7.40863	0	
20	k__Bacteria|p__Bacteroide	0	0	0	0	0	0	0.00281	0	0	0	0.13473	1.12503	0	0.01746	0.00159	0.0094
21	k__Bacteria|p__Actinobact	0	0.07358	0.71281	0	0	0	0	0	0	0	0	0	10.69807	1.55503	0	
22	k__Bacteria|p__Firmicutes	8e-05	0.53975	0.70681	0.02233	0	0	0	0	0	0	0	0	6.75115	0.29668	0	
23	k__Bacteria|p__Proteobact	0	0.0049	1.10502	0	0	0	0	0	0	0	0	0	0.23766	0.12036	0.01257	
24	k__Bacteria|p__Firmicutes	0.00075	0.00467	1.79514	0.04706	0	0	0.00087	0	0	0	0	0	0.75602	0.47212	0	
25	k__Bacteria|p__Fusobacte	0	0.07934	0.03212	0	0	0	0	0	0	0	0	0	0.0556	0.00685	0	
26	k__Bacteria|p__Bacteroide	0	0	0	0.01024	0	0.00011	0.0001	0	0	0	0	0.019	0	0	0	
27	k__Bacteria|p__Verrucomic	0	0	0	1.92544	0	0.00249	0	0	0	0	0	5.01912	0	0.03603	0	1.9544
28	k__Bacteria|p__Fusobacte	0	0.18413	0.09554	0	0	0	0	0	0	0	0	0	0.13772	0.05674	0	
29	k__Bacteria|p__Bacteroide	84.60804	0.17674	1.20942	58.53925	0	87.17536	80.23906	0	0	0.00528	95.18632	90.48074	0.89671	6.19701	90.3718	93.8067
30	k__Bacteria|p__Proteobact	0	0.0049	1.10502	0	0	0	0	0	0	0	0	0	0.23766	0.12036	0.01257	
31	k__Bacteria|p__Firmicutes	0	3.7702	5.24454	0	0	0	0	0	0	0	0.00317	0	5.2466	20.28856	0	
32	k__Bacteria|p__Firmicutes	0	0	0	0	28.41152	0	0	0	98.62158	0.09156	0	0	0	0	0	
33	k__Bacteria|p__Bacteroide	0	0	0	0.01284	0	0.00127	0.00134	0	0	0	0	0	0	0	0	2.3011
34	k__Bacteria|p__Bacteroide	0	0	0	0.13547	0	0.66733	0.93226	0	0	0	1.02811	0	0	0.1132	0	1.5792
35	k__Bacteria|p__Firmicutes	0	0	0	0	0	0	3.28129	0	0	0	0	0	0	0	0	
36	k__Bacteria|p__Actinobact	0	0	0	0.0256	0	0.15669	0	0	0	0	0	0	0	0.02756	0.50559	
37	k__Bacteria|p__Firmicutes	15.11446	0	0.22313	31.85621	0.01935	3.9669	13.62363	0	0	0.01816	4.1026	3.84111	0.18439	9.12663	5.54894	3.5452
38	k__Bacteria|p__Firmicutes	0.00108	11.78992	0.19295	0.00324	0	0	0	0	0	0	0	0	1.76326	0.7472	0	
39	k__Bacteria|p__Proteobact	0.06223	0	0	0.01538	0	0.00569	0	0	0	0	0.0034	0	0	0.01972	0.29636	
40	k__Bacteria|p__Bacteroide	2.57633	0	0	3.72212	0	0.47934	1.40935	0	0	0	0	0.65938	0	0.59358	2.11564	1.4296

Sheet1

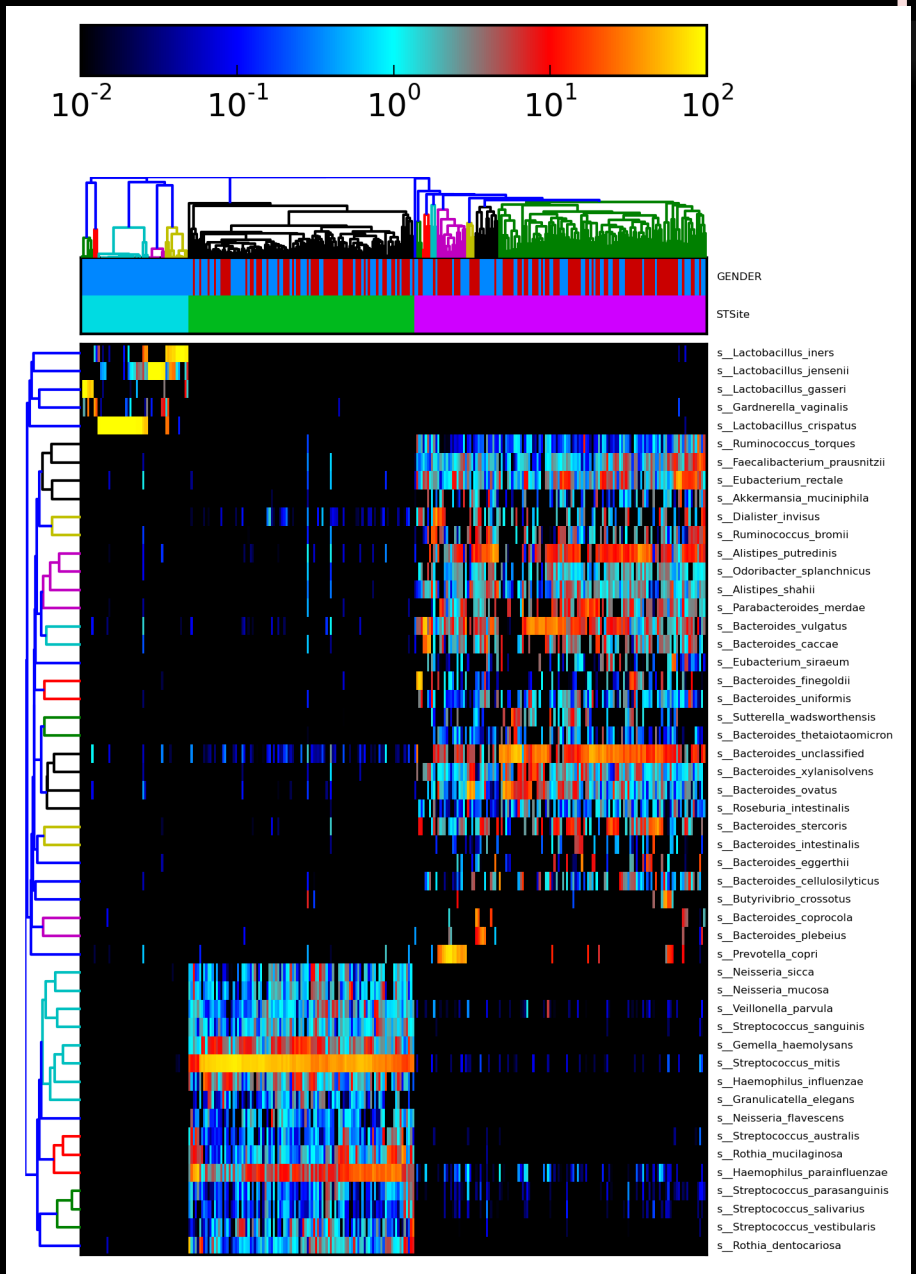
Sheet 1 / 1 Default STD CountA=1 100%

Generate heatmap with hclust2

```
cat HMP.ab.filtered.metadata.tsv | grep -E 'sid|GENDER|
STSite|s__' | grep -v "t__" | sed "s/.*|//" >
HMP.ab.filtered.metadata.txt

ln -s ~/biobakery/hclust2/hclust2.py

hclust2.py -h | less

hclust2.py -i HMP.ab.filtered.metadata.txt -o
HMP.log_scale.png  --ftop 50 --f_dist_f correlation --
s_dist_f braycurtis --cell_aspect_ratio 9 -l --fperc 99
--flabel_size 4 --metadata_rows 1,2 --legend_file
HMP.log_scale.legend.png --max_flabel_len 100 --
metadata_height 0.075 --minv 0.01 --no_slabels --dpi
300
```

https://bitbucket.org/nsegata/hclust2

Generate heatmap with hclust2

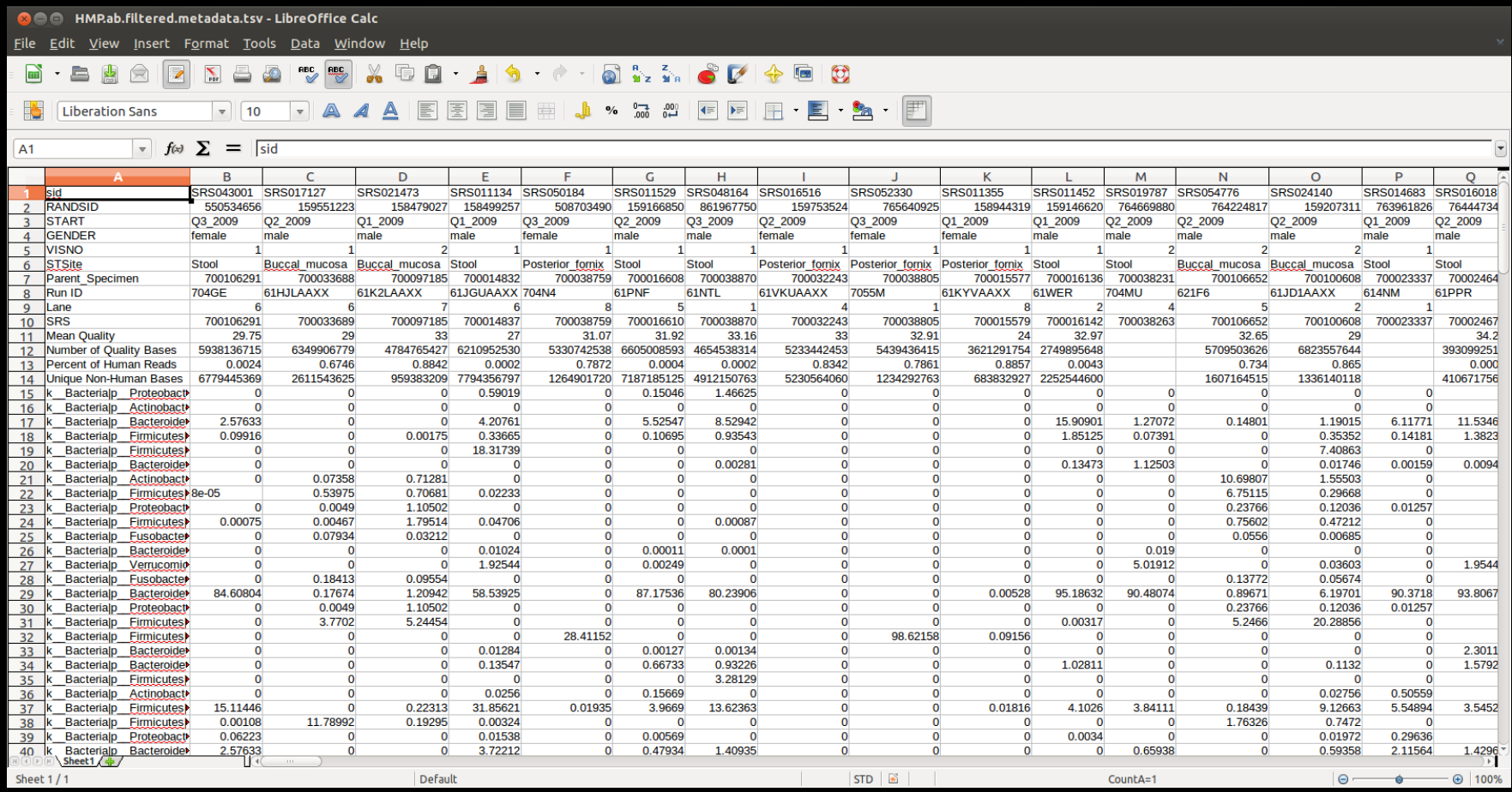


GENDER
male
female

STSite
Buccal_mucosa
Stool
Posterior_fornix

What it means: LEfSe

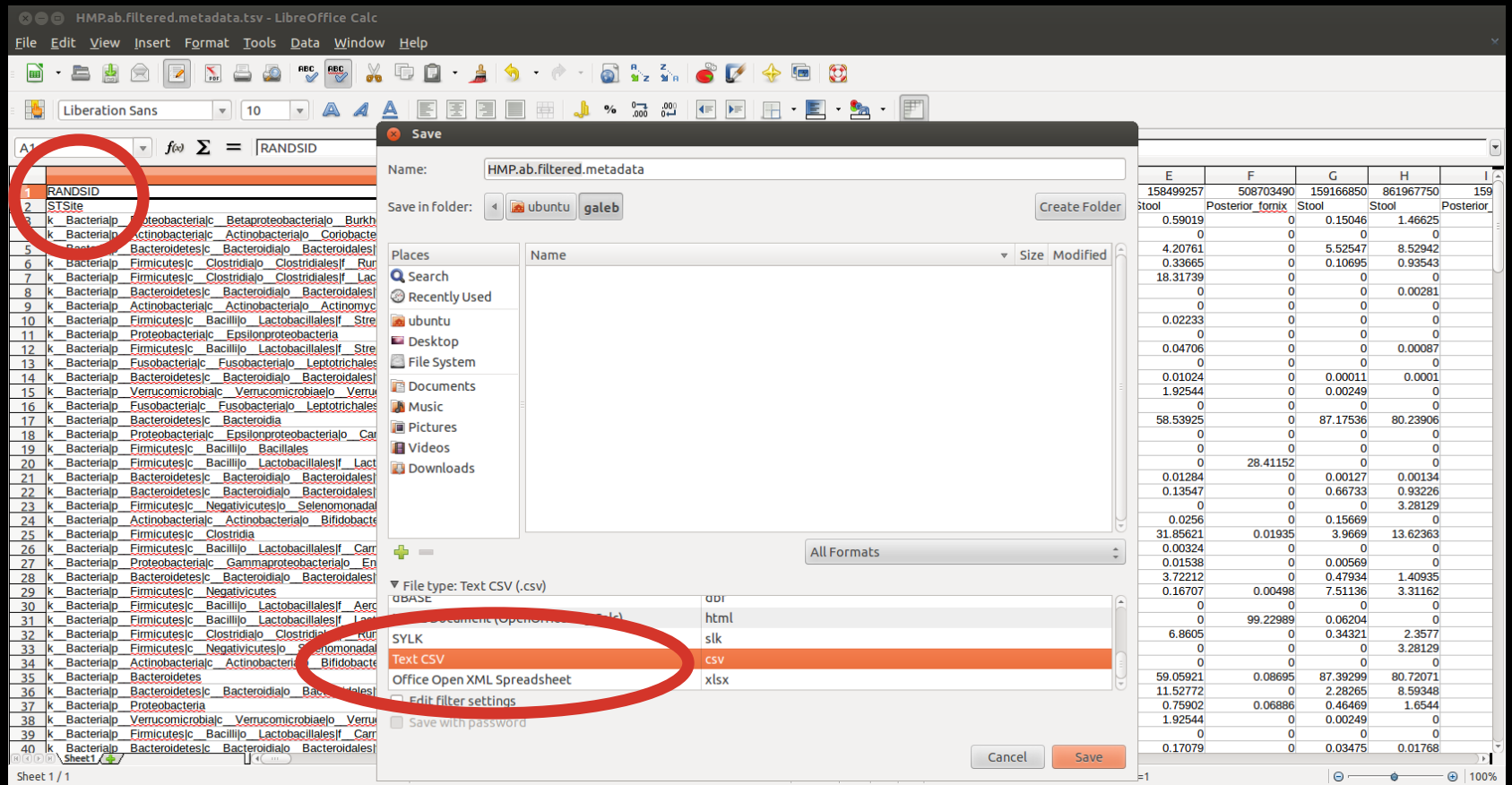
- Let's modify the *.tsv file to be for LEfSe



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	sid	SRS043001	SRS017127	SRS021473	SRS011134	SRS050184	SRS011529	SRS048164	SRS016516	SRS052330	SRS011355	SRS011452	SRS019787	SRS054776	SRS024140	SRS014683	SRS016018
2	RANDSID	550534656	159551223	158479027	158499257	508703490	159166850	861967750	159753524	765640925	158944319	159146620	764669880	764224817	159207311	763961826	76444734
3	START	Q3_2009	Q2_2009	Q1_2009	Q1_2009	Q3_2009	Q2_2009	Q3_2009	Q2_2009	Q3_2009	Q1_2009	Q1_2009	Q2_2009	Q2_2009	Q2_2009	Q1_2009	Q2_2009
4	GENDER	female	male	male	male	female	male	male	female	female	female	male	male	male	male	male	male
5	VISNO	1	1	2	1	1	1	1	1	1	1	1	2	2	2	1	
6	STSite	Stool	Buccal_mucosa	Buccal_mucosa	Stool	Posterior_fornix	Stool	Stool	Posterior_fornix	Posterior_fornix	Posterior_fornix	Stool	Stool	Buccal_mucosa	Buccal_mucosa	Stool	Stool
7	Parent_Specimen	700106291	700033688	700097185	700014832	700038759	700016608	700038870	700032243	700038805	700015577	700016136	700038231	700106652	700100608	700023337	70002464
8	Run ID	704GE	61HJLAAXX	61K2LAAXX	61JGUAAXX	704N4	61PNF	61NTL	61VKUAAXX	7055M	61KYVAAXX	61WER	704MU	621F6	61JD1AAXX	614NM	61PPR
9	Lane	6	6	7	6	8	5	1	4	1	8	2	4	5	2	1	
10	SRS	700106291	700033689	700097185	700014837	700038759	700016610	700038870	700032243	700038805	700015579	700016142	700038263	700106652	700100608	700023337	70002467
11	Mean Quality	29.75	29	33	27	31.07	31.92	33.16	33	32.91	24	32.97		32.65	29		34.2
12	Number of Quality Bases	5938136715	6349906779	4784765427	6210952530	5330742538	6605008593	4654538314	5233442453	5439436415	3621291754	2749895648		5709503626	6823557644		393099251
13	Percent of Human Reads	0.0024	0.6746	0.8842	0.0002	0.7872	0.0004	0.0002	0.8342	0.7861	0.8857	0.0043		0.734	0.865		0.000
14	Unique Non-Human Bases	6779445369	2611543625	959383209	7794356797	1264901720	7187185125	4912150763	5230564060	1234292763	683832927	2252544600		1607164515	1336140118		410671756
15	k__Bacteria|p__Proteobact	0	0	0	0.59019	0	0.15046	1.46625	0	0	0	0	0	0	0	0	
16	k__Bacteria|p__Actinobact	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	k__Bacteria|p__Bacteroide	2.57633	0	0	4.20761	0	5.52547	8.52942	0	0	0	15.90901	1.27072	0.14801	1.19015	6.11771	11.5346
18	k__Bacteria|p__Firmicutes	0.09916	0	0.00175	0.33665	0	0.10695	0.93543	0	0	0	1.85125	0.07391	0	0.35352	0.14181	1.3823
19	k__Bacteria|p__Firmicutes	0	0	0	18.31739	0	0	0	0	0	0	0	0	0	7.40863	0	
20	k__Bacteria|p__Bacteroide	0	0	0	0	0	0	0.00281	0	0	0	0.13473	1.12503	0	0.01746	0.00159	0.0094
21	k__Bacteria|p__Actinobact	0	0.07358	0.71281	0	0	0	0	0	0	0	0	0	10.69807	1.55503	0	
22	k__Bacteria|p__Firmicutes	8e-05	0.53975	0.70681	0.02233	0	0	0	0	0	0	0	0	6.75115	0.29668	0	
23	k__Bacteria|p__Proteobact	0	0.0049	1.10502	0	0	0	0	0	0	0	0	0	0.23766	0.12036	0.01257	
24	k__Bacteria|p__Firmicutes	0.00075	0.00467	1.79514	0.04706	0	0	0.00087	0	0	0	0	0	0.75602	0.47212	0	
25	k__Bacteria|p__Fusobacte	0	0.07934	0.03212	0	0	0	0	0	0	0	0	0	0.0556	0.00685	0	
26	k__Bacteria|p__Bacteroide	0	0	0	0.01024	0	0.00011	0.0001	0	0	0	0	0.019	0	0	0	
27	k__Bacteria|p__Verrucomi	0	0	0	1.92544	0	0.00249	0	0	0	0	0	5.01912	0	0.03603	0	1.9544
28	k__Bacteria|p__Fusobacte	0	0.18413	0.09554	0	0	0	0	0	0	0	0	0	0.13772	0.05674	0	
29	k__Bacteria|p__Bacteroide	84.60804	0.17674	1.20942	58.53925	0	87.17536	80.23906	0	0	0	0.00528	95.18632	90.48074	0.89671	6.19701	90.3718	93.8067
30	k__Bacteria|p__Proteobact	0	0.0049	1.10502	0	0	0	0	0	0	0	0	0	0.23766	0.12036	0.01257	
31	k__Bacteria|p__Firmicutes	0	3.7702	5.24454	0	0	0	0	0	0	0	0	0.00317	0	5.2466	20.28856	0	
32	k__Bacteria|p__Firmicutes	0	0	0	0	28.41152	0	0	0	0	98.62158	0.09156	0	0	0	0	0	
33	k__Bacteria|p__Bacteroide	0	0	0	0.01284	0	0.00127	0.00134	0	0	0	0	0	0	0	0	2.3011
34	k__Bacteria|p__Bacteroide	0	0	0	0.13547	0	0.66733	0.93226	0	0	0	1.02811	0	0	0.1132	0	1.5792
35	k__Bacteria|p__Firmicutes	0	0	0	0	0	0	3.28129	0	0	0	0	0	0	0	0	
36	k__Bacteria|p__Actinobact	0	0	0	0.0256	0	0.15669	0	0	0	0	0	0	0	0.02756	0.50559	
37	k__Bacteria|p__Firmicutes	15.11446	0	0.22313	31.85621	0.01935	3.9669	13.62363	0	0	0	0.01816	4.1026	3.84111	0.18439	9.12663	5.54894	3.5452
38	k__Bacteria|p__Firmicutes	0.00108	11.78992	0.19295	0.00324	0	0	0	0	0	0	0	0	1.76326	0.7472	0	
39	k__Bacteria|p__Proteobact	0.06223	0	0	0.01538	0	0.00569	0	0	0	0	0.0034	0	0	0.01972	0.29636	
40	k__Bacteria|p__Bacteroide	2.57633	0	0	3.72212	0	0.47934	1.40935	0	0	0	0	0.65938	0	0.59358	2.11564	1.4296

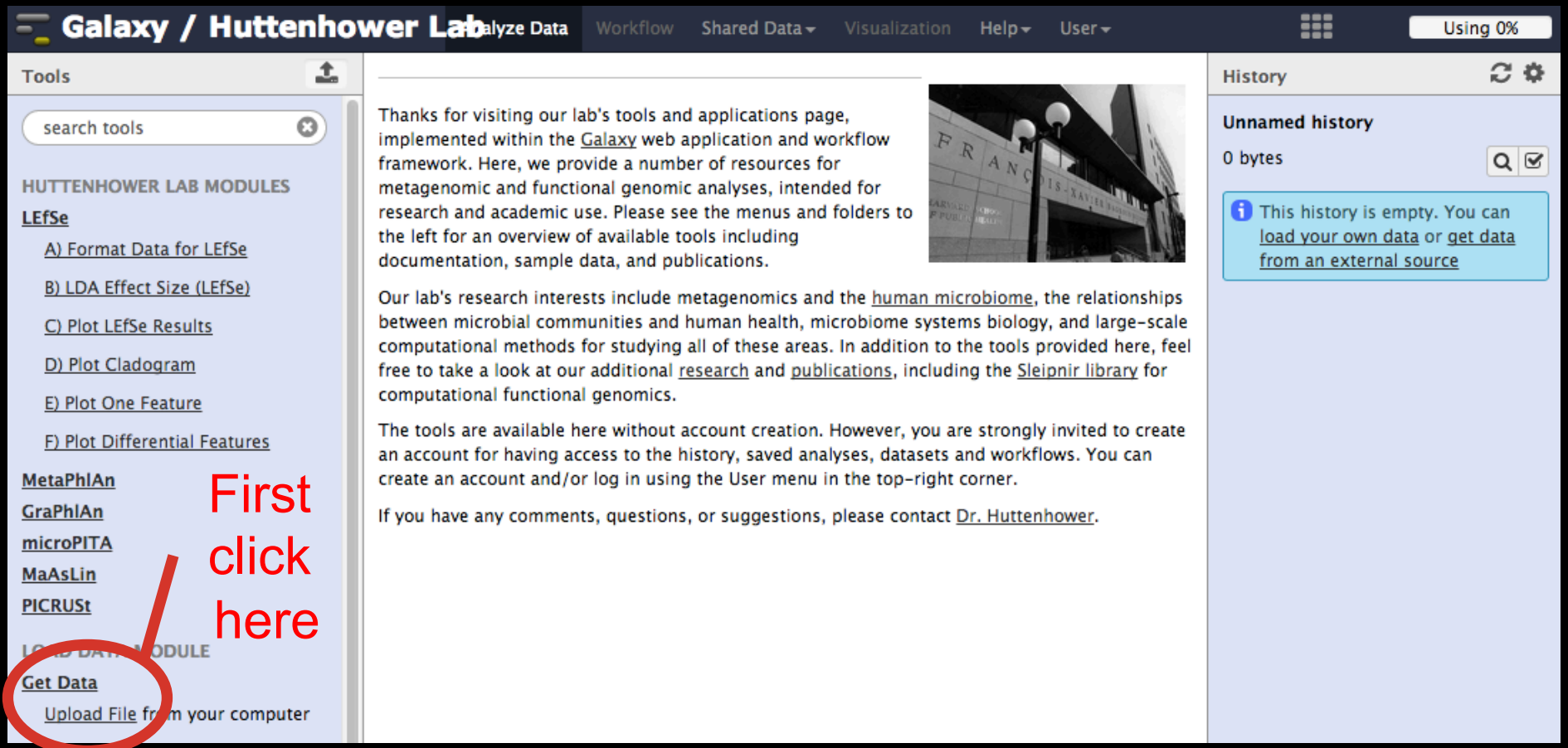
What it means: LEfSe

- Delete all of the metadata rows *except*:
  - RANDSID and STSite
  - Save it as tab-delimited text: `HMP.ab.filtered.metadata2.txt`



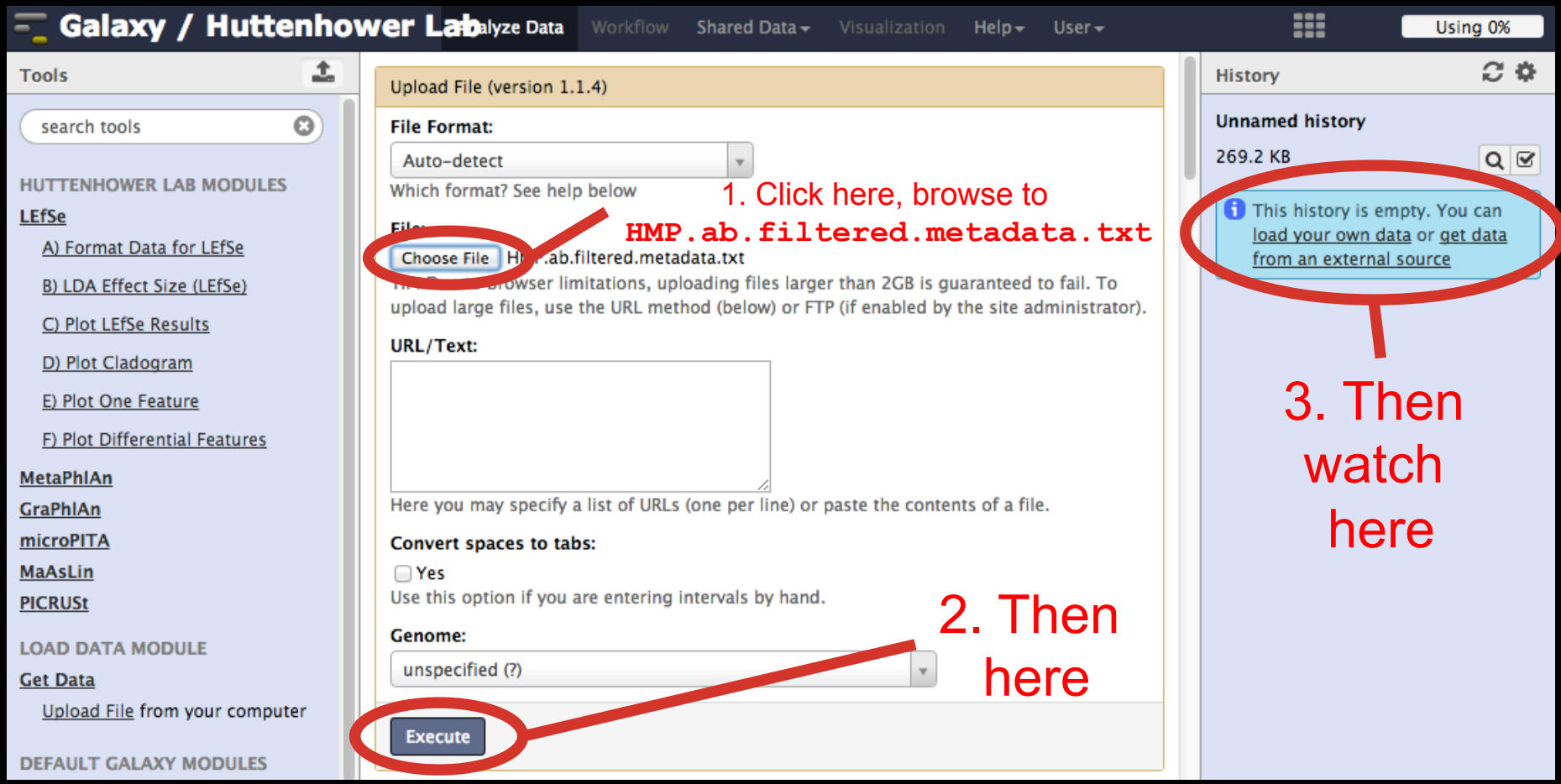
What it means: LEfSe

- Visit LEfSe at: http://huttenhower.sph.harvard.edu/galaxy/



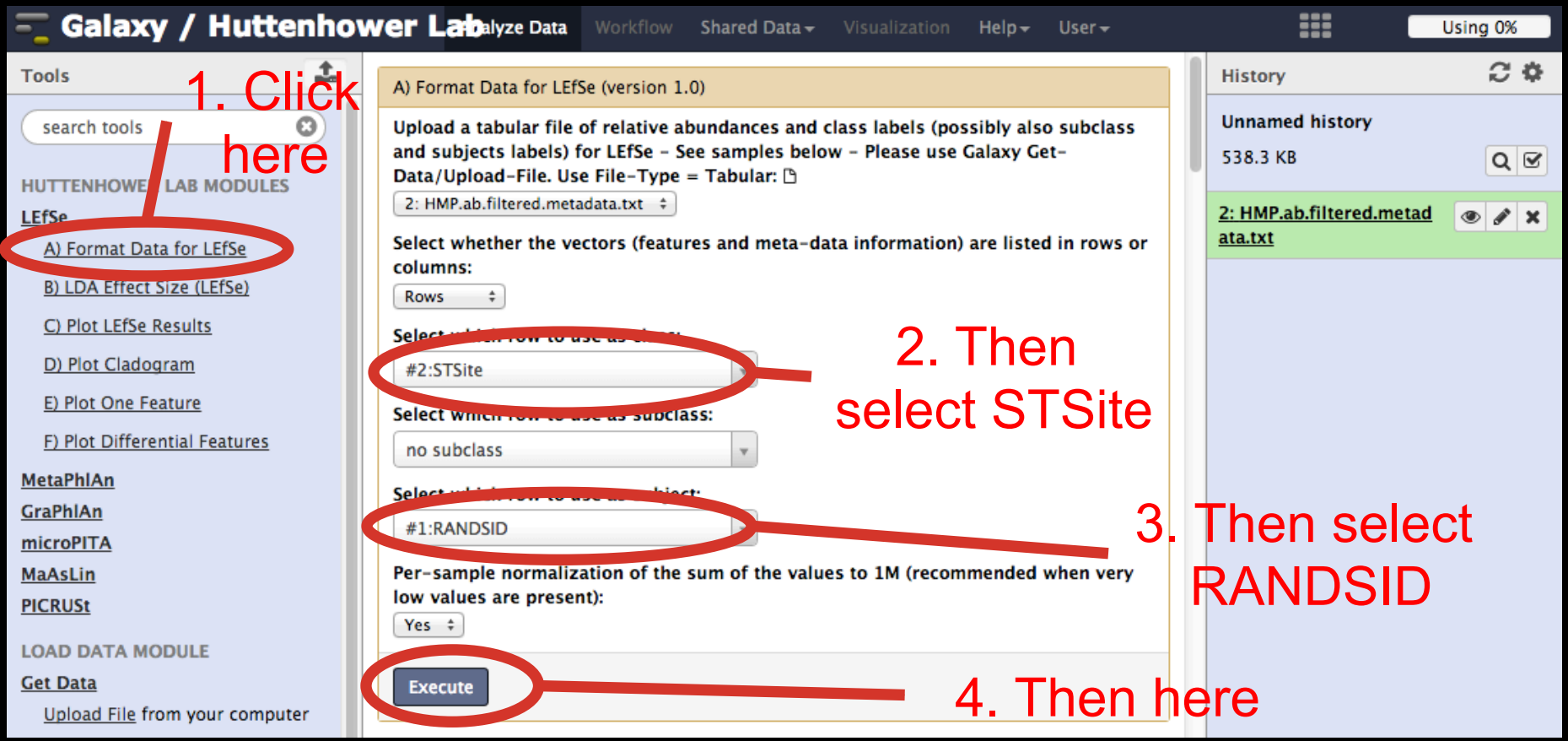
What it means: LEfSe

- Then upload your formatted table
  - After you upload, wait for the progress meter to turn green!



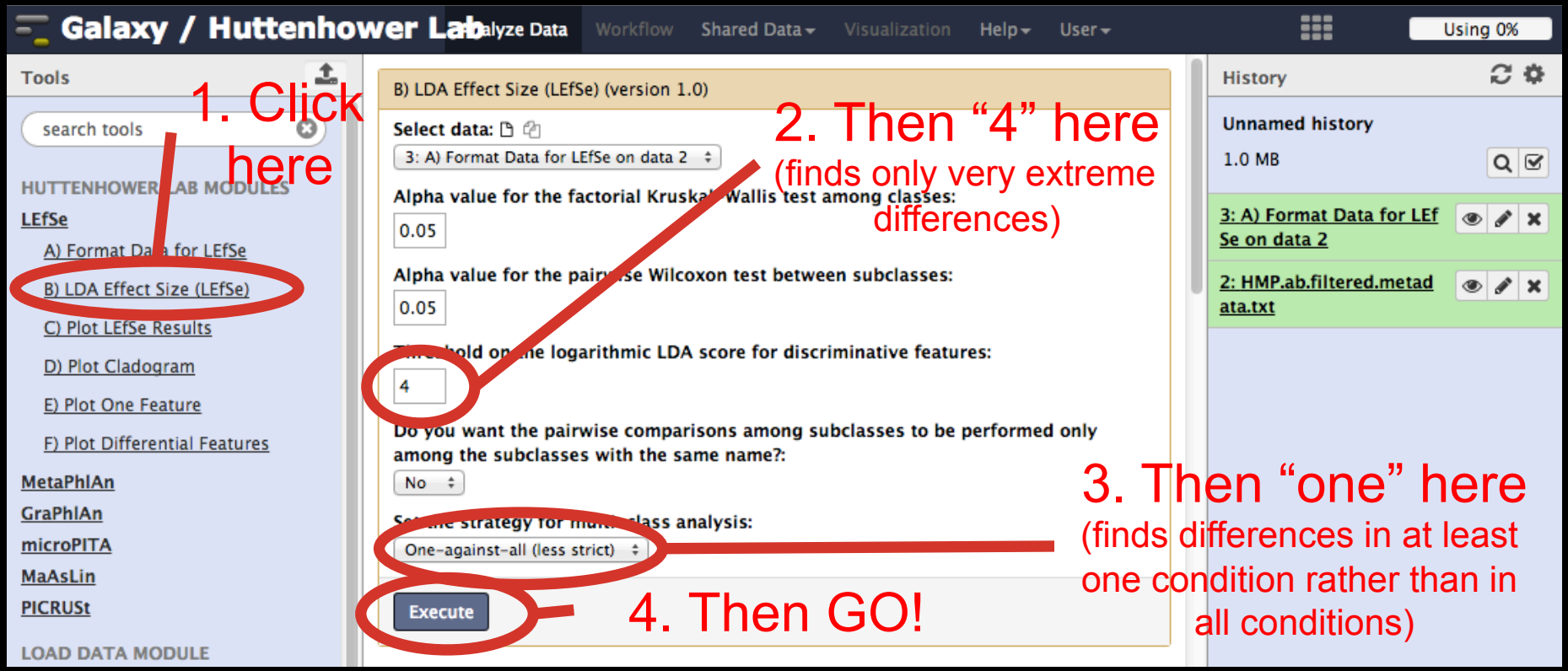
What it means: LEfSe

- Then tell LEfSe about your metadata:



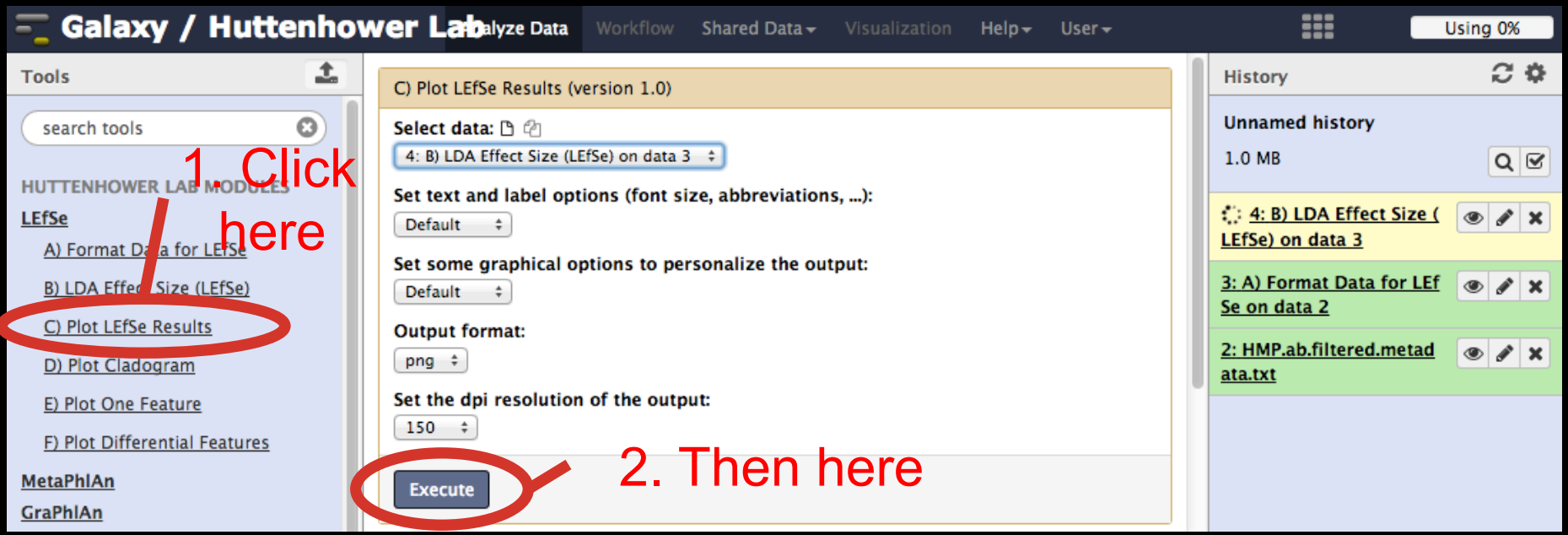
What it means: LEfSe

- Then select LDA=4, “One-against-all,” and run LEfSe!
  - You can change other default statistical parameters if desired



What it means: LEfSe

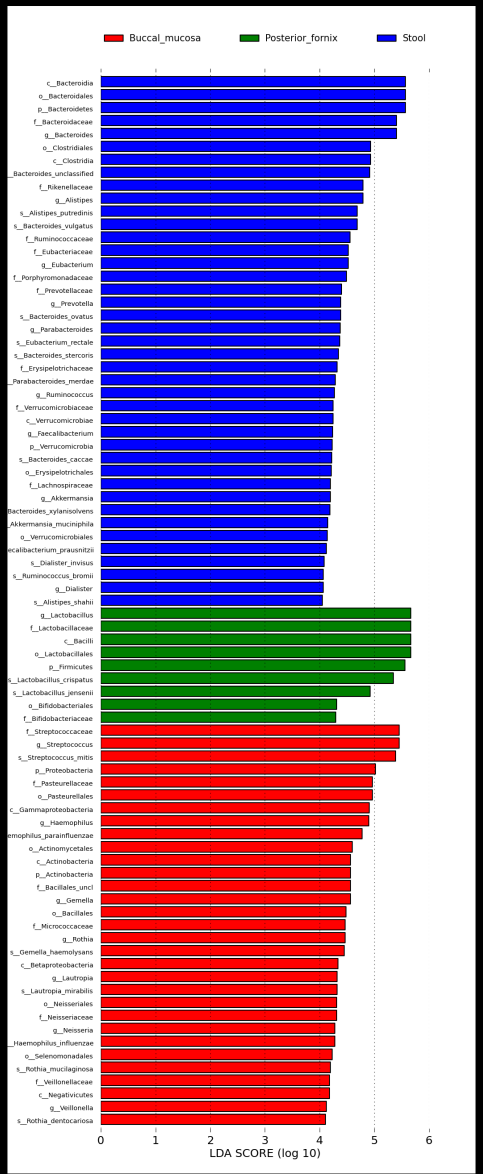
- You can plot the results as a bar plot
  - Again, lots of graphical parameters to modify if desired



What it means: LEfSe

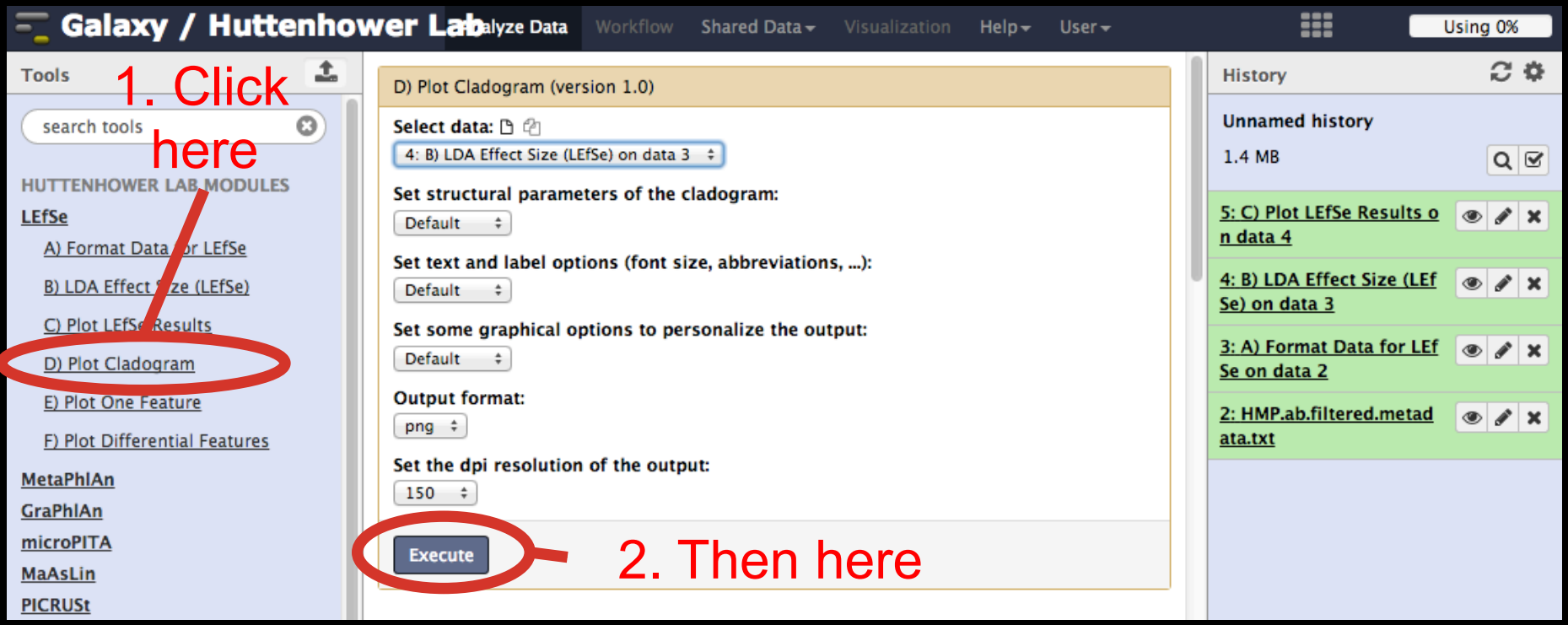
- In Galaxy, view a result by clicking on its “eye”

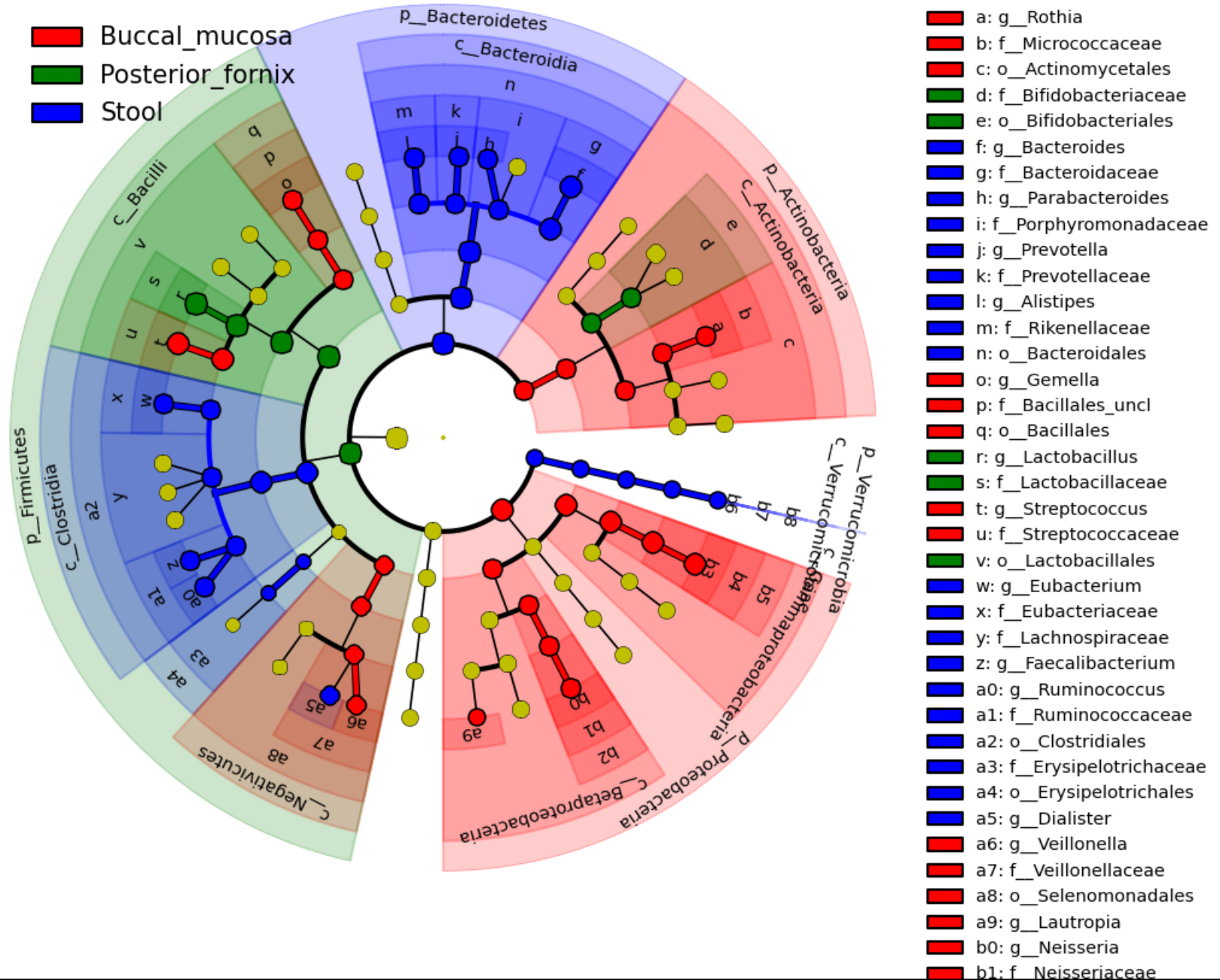
What it means: LEfSe



What it means: LEfSe

- You can plot the results as a cladogram
  - Lots and *lots* of graphical parameters to modify if desired

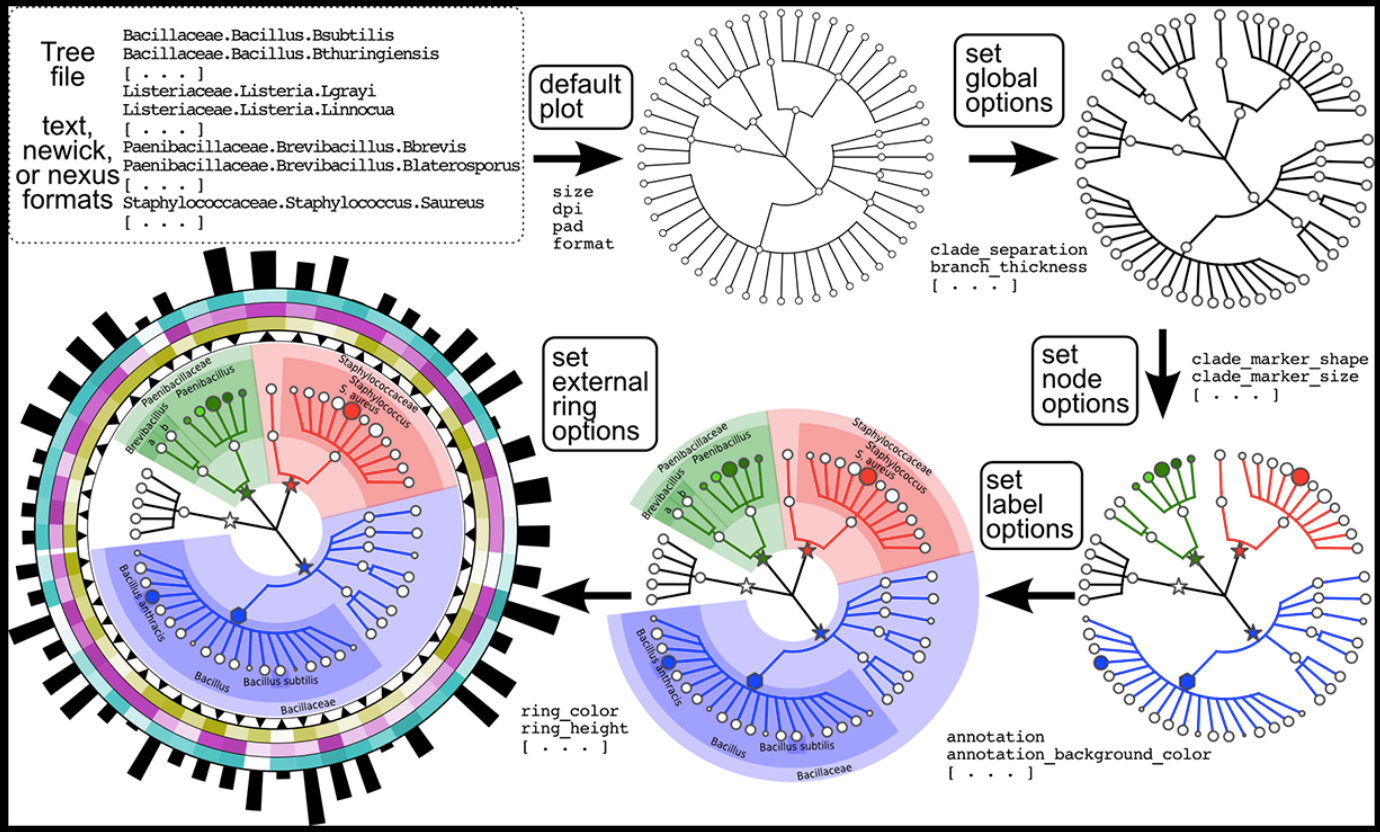



Buccal_mucosa
Posterior_fornix
Stool
p__Bacteroidetes
c__Bacteroidia
p__Actinobacteria
c__Actinobacteria
p__Firmicutes
c__Bacilli
c__Clostridia
a2
c__Negativicutes
c__Betaproteobacteria
p__Proteobacteria
c__Gammaproteobacteria
p__Verrucomicrobia
c__Verrucomicrobiae
a: g__Rothia
b: f__Micrococcaceae
c: o__Actinomycetales
d: f__Bifidobacteriaceae
e: o__Bifidobacteriales
f: g__Bacteroides
g: f__Bacteroidaceae
h: g__Parabacteroides
i: f__Porphyromonadaceae
j: g__Prevotella
k: f__Prevotellaceae
l: g__Alistipes
m: f__Rikenellaceae
n: o__Bacteroidales
o: g__Gemella
p: f__Bacillales_uncl
q: o__Bacillales
r: g__Lactobacillus
s: f__Lactobacillaceae
t: g__Streptococcus
u: f__Streptococcaceae
v: o__Lactobacillales
w: g__Eubacterium
x: f__Eubacteriaceae
y: f__Lachnospiraceae
z: g__Faecalibacterium
a0: g__Ruminococcus
a1: f__Ruminococcaceae
a2: o__Clostridiales
a3: f__Erysipelotrichaceae
a4: o__Erysipelotrichales
a5: g__Dialister
a6: g__Veillonella
a7: f__Veillonellaceae
a8: o__Selenomonadales
a9: g__Lautropia
b0: g__Neisseria
b1: f__Neisseriaceae

An aside: GraPhlAn

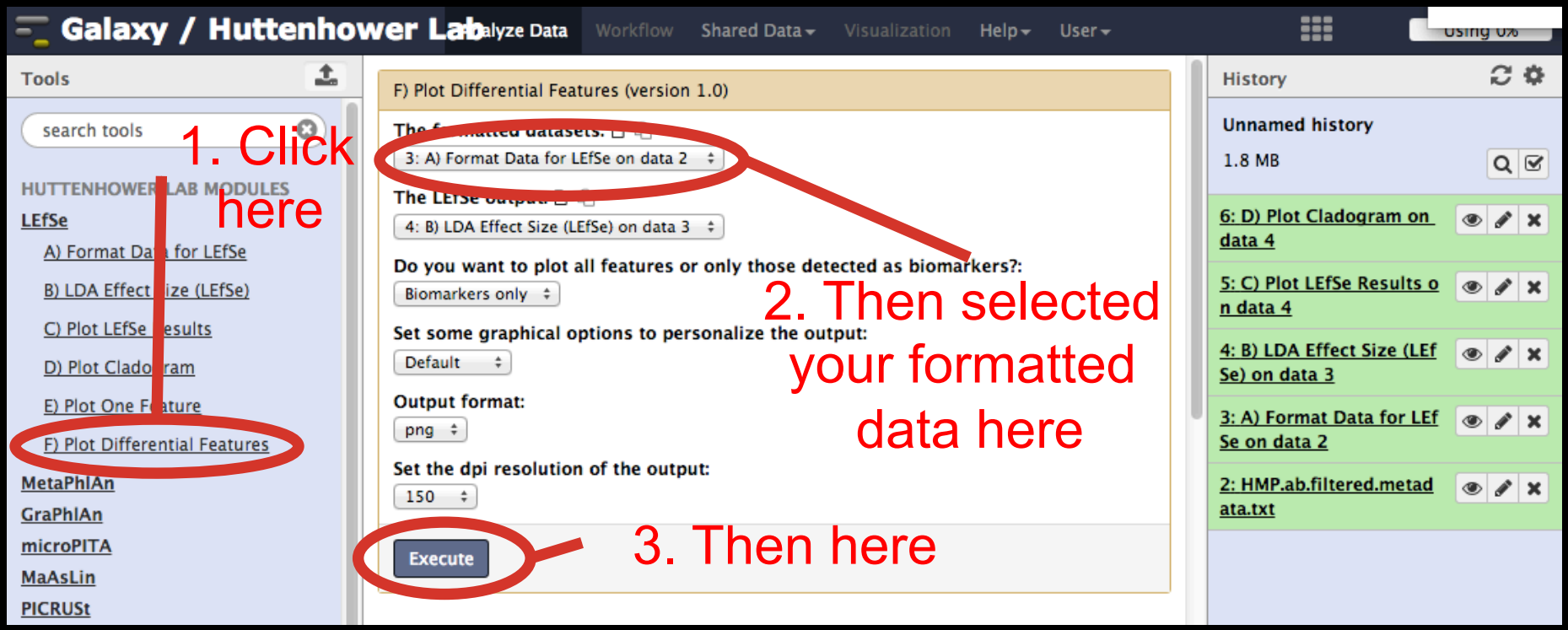
- You can use this visualization for other purposes as well
  - Available online through Galaxy
  - Available offline as open source Python

http://huttenhower.sph.harvard.edu/graphlan



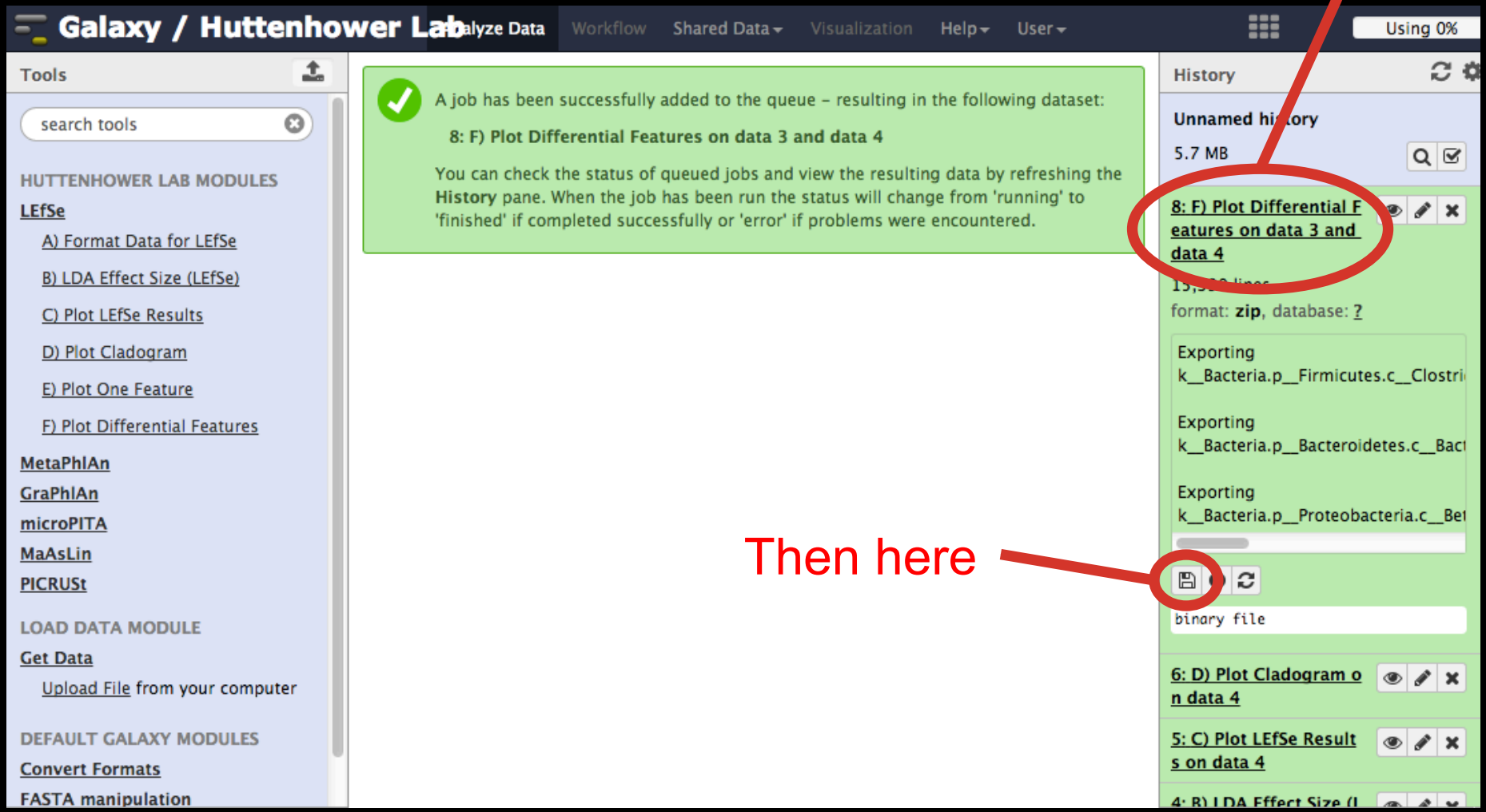
What it means: LEfSe

- Finally, you can see the raw data for individual biomarkers
  - These are generated as a zip file of individual plots



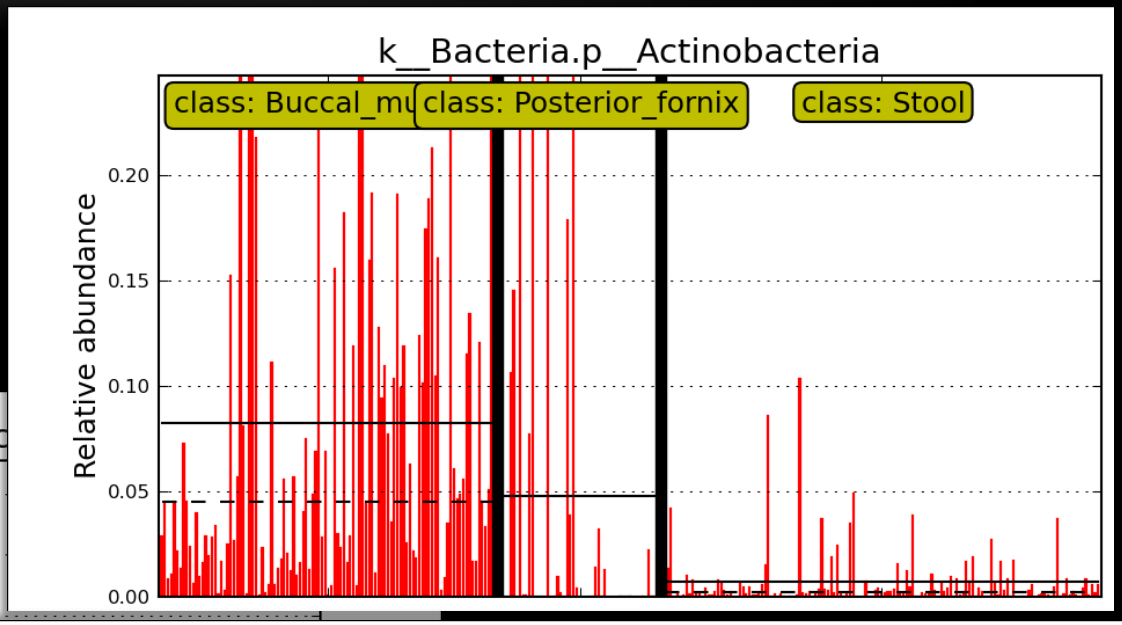
What it means: LEfSe

- In Galaxy, download a result by clicking on its “disk”

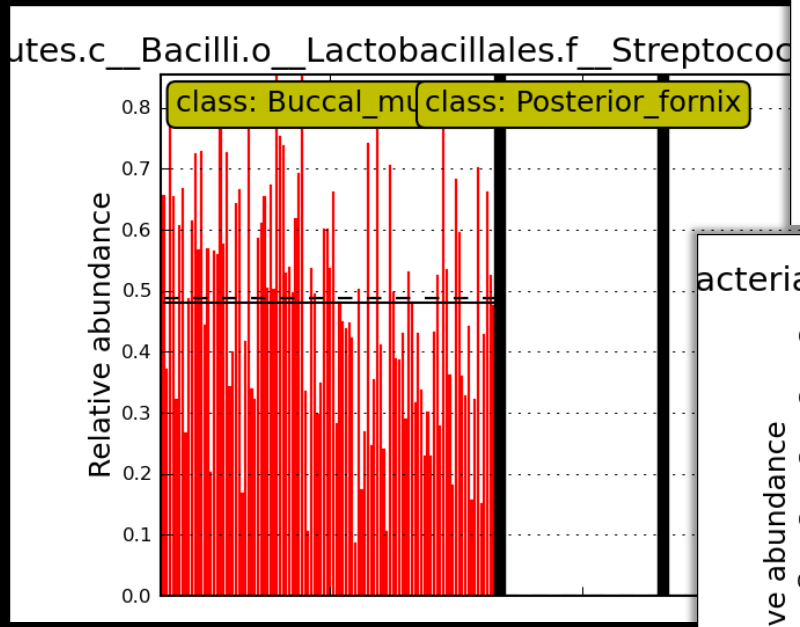


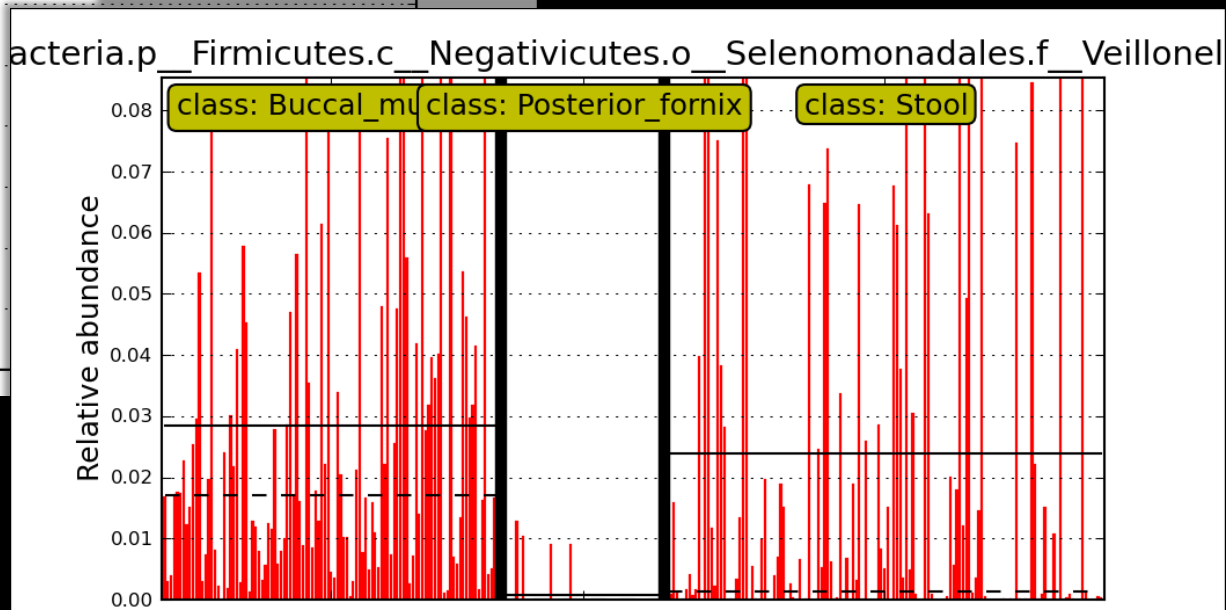
What it means: LEfSe

Actinobacteria



Strep. mitis





Veillonellaceae

Summary

- MetaPhlAn2
  - Evolution of MetaPhlAn1
    - Viruses, euks, subspecies, speed
    - And a LOT more reference data!
  - Raw metagenomic reads in
  - Tab-delimited species relative abundances out

- LEfSe
  - Tab-delimited, stratified relative abundances in
  - Significantly differentially abundant features out

Meta’omic functional profiling with ShortBRED

The two big questions…

Who is there?
(taxonomic profiling)

What are they doing?
(functional profiling)

What's there: ShortBRED

Jim Kaminski

- **ShortBRED** is a tool for quantifying protein families in metagenomes
  - Short Better REad Dataset

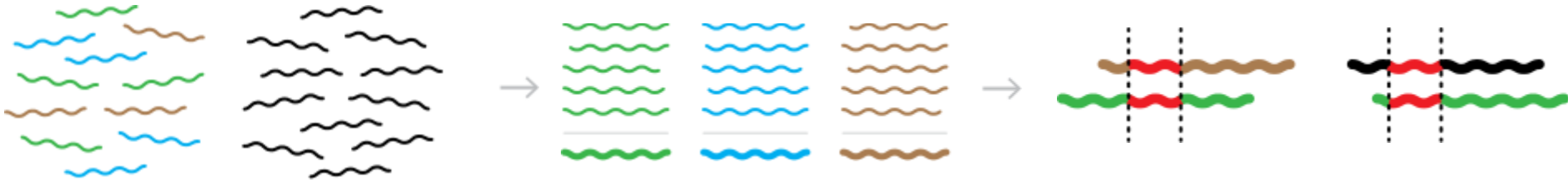
- Inputs:
  - FASTA file of proteins of interest
  - Large reference database of protein sequences (FASTA or blastdb)
  - Metagenomes (FASTA/FASTQ nucleotide files)
- Outputs:
  - Short, unique markers for protein families of interest (FASTA)
  - Relative abundances of protein families of interest in each metagenome (text file, RPKM)

- Compared to BLAST (or HUMAnN), this is:
  - Faster
  - More specific

What's there: ShortBRED algorithm

- Cluster proteins of interest into families
  - Record consensus sequences
- Identify and common areas among proteins
  - Compared against each other
  - Compared against reference database
  - Remove all of these
- Remaining subseqs. uniquely ID a family
  - Record these as markers for that family

What's there: ShortBRED marker identification

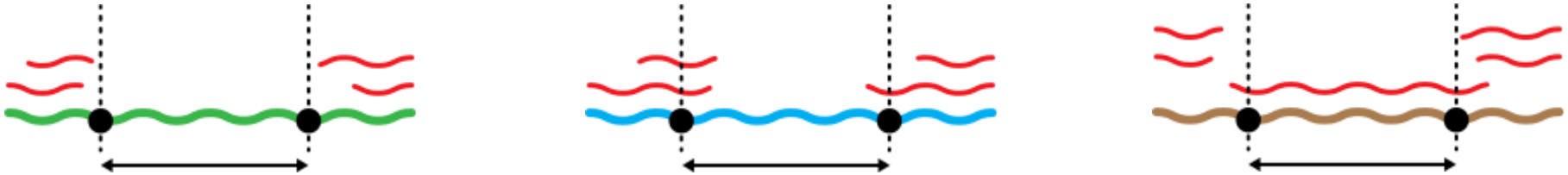


Prots of interest

Reference database

Cluster into families

Identify short, common regions

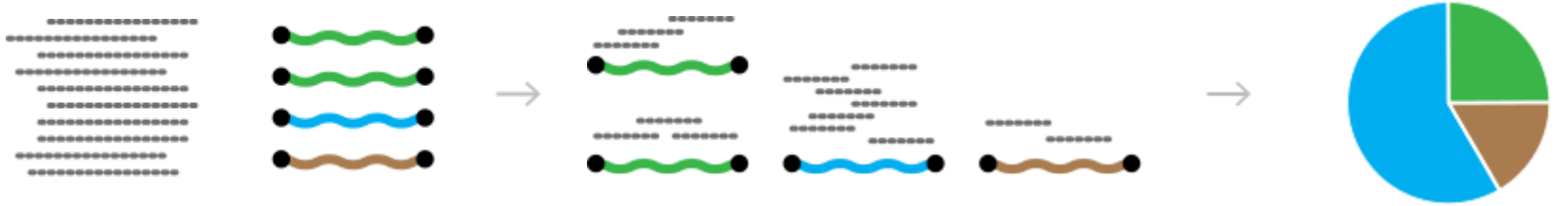


True Marker

Junction Marker

Quasi Marker

What's there: ShortBRED family quantification



Metagenome reads

ShortBRED markers

Translated search for high ID hits

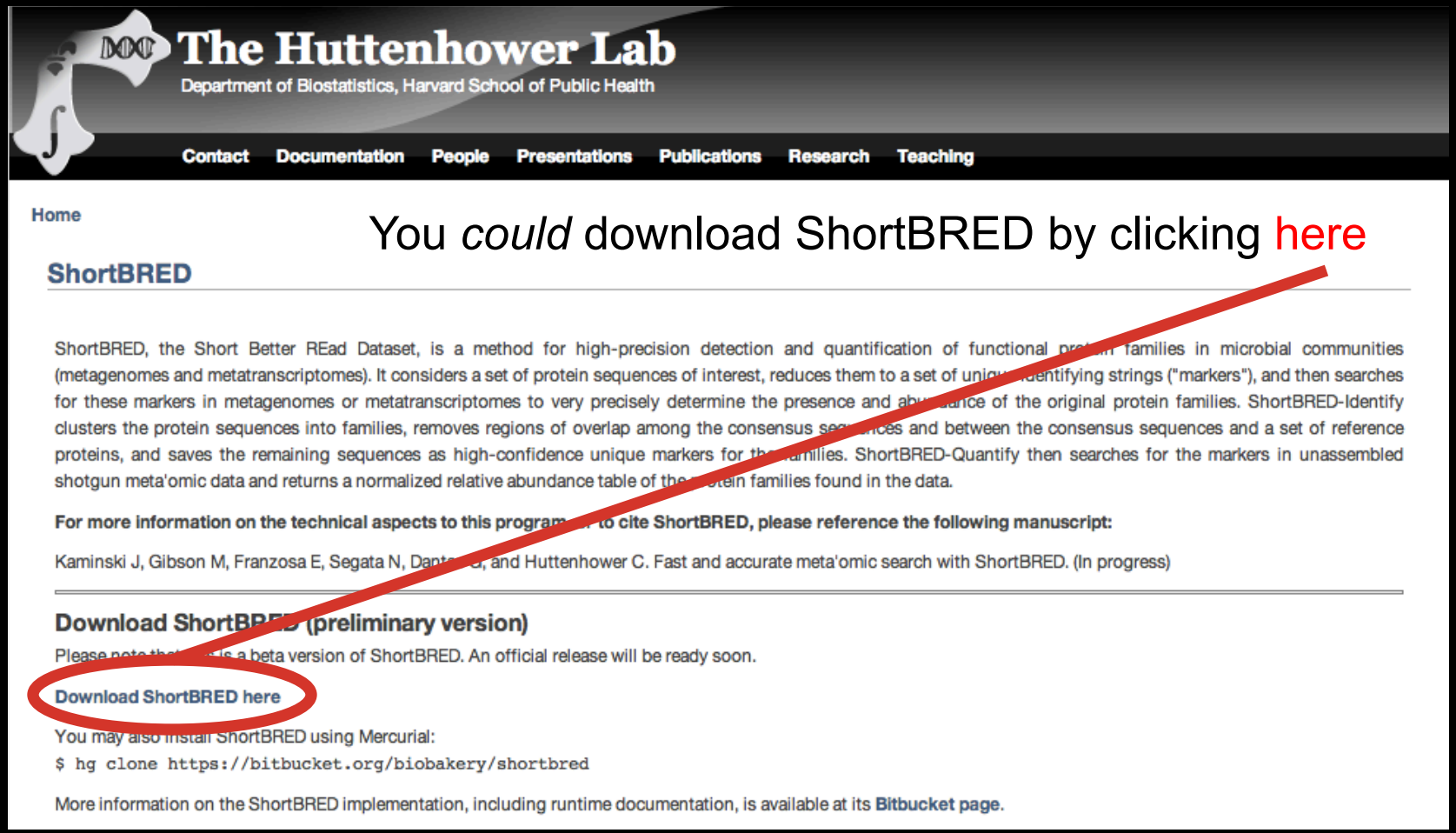
Normalize relative abundances

Setup notes reminder

- Slides with green titles or text include instructions not needed today, but useful for your own analyses
- Keep an eye out for red warnings of particular importance
- Command lines and program/file names appear in a `monospaced font`.
- Commands you should specifically copy/paste are in **`monospaced bold blue`**.

What's there: ShortBRED

- ShortBRED is available at http://huttenhower.sph.harvard.edu/shortbred



From the command line...

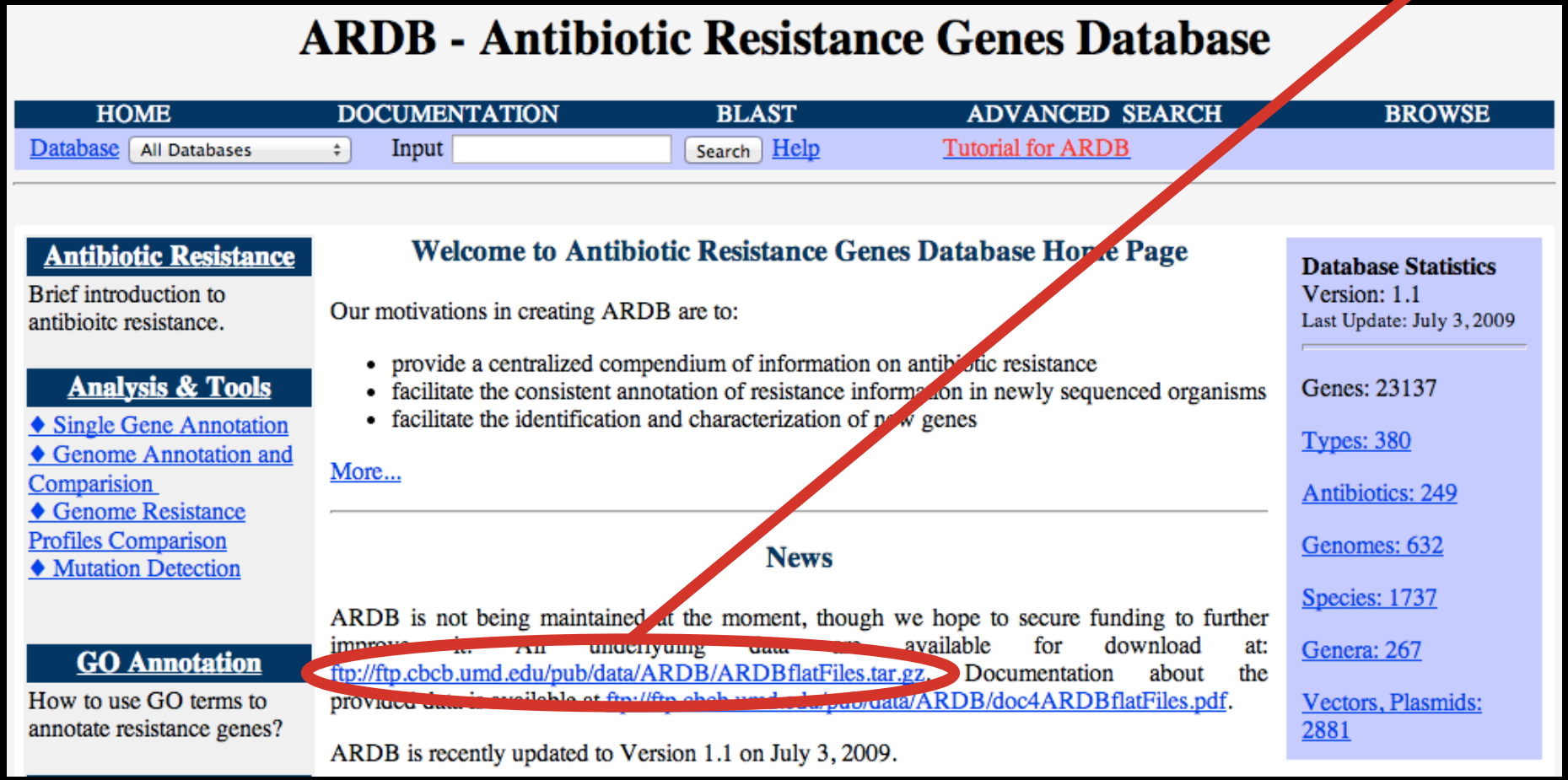
- But don't!
  - Instead, we've installed ShortBRED already for you
- To see what you can do, run:

```
shortbred_identify.py -h | less

shortbred_quantify.py -h | less
```

Getting some annotated protein sequences

You could download the ARDB protein sequences here

- Go to http://ardb.cbcb.umd.edu



From the command line...

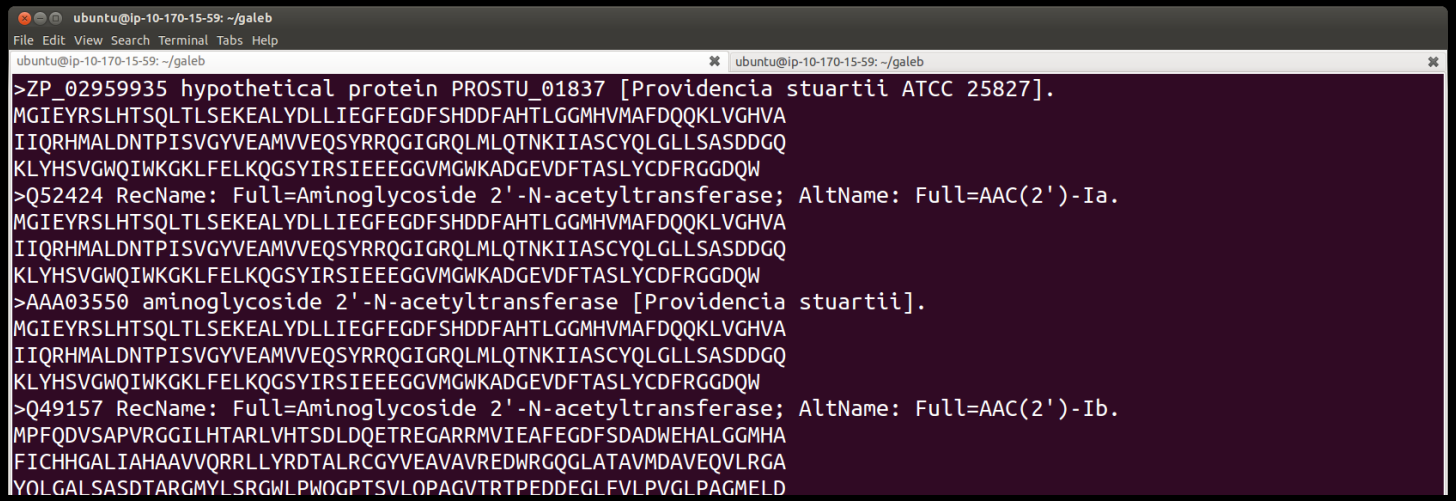
- But don't!
  - Instead, we've downloaded the important file for you

```
ln -s /home/ubuntu/biobakery/shortbred/data/resisGenes.pfasta
```

```
ln -s /home/ubuntu/biobakery/shortbred/data/resisGenes.pfasta
```

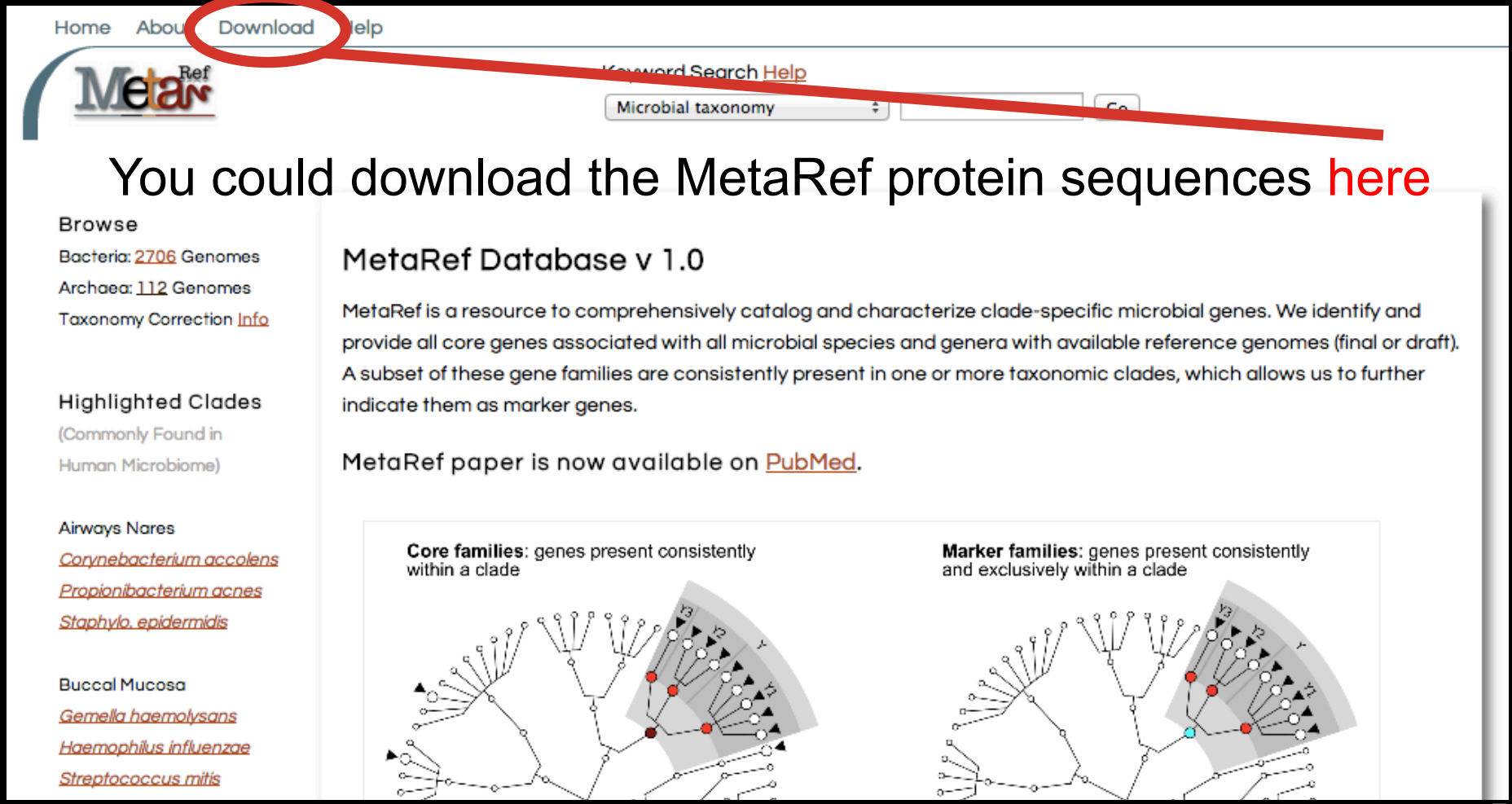
- Take a look by running:

```
less resisGenes.pfasta
```



Getting some reference protein sequences

- Go to http://metaref.org



Running ShortBRED-Identify

- But don't!
  - We'll use an example mini reference database for speed
- Lets make some antibiotic resistance markers by running:

```
shortbred_identify.py --goi resisGenes.pfasta
--ref ref_prots.faa --markers ardb_markers.faa
```

  - This should take ~5 minutes
    - If you get bored waiting, kill it and copy:

```
/class/stamps-shared/biobakery/results/shortbred/ardb_markers.faa
```

  - It will produce lots of status output as it runs

```
less ardb_markers.faa
```

ShortBRED markers

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Help

```
>ZP_01723236_TM_#01
TEEFLGKYP
>ZP_01723236_TM_#02
IVVMWKRMLSLVGLYKIDGQSQSINRRFNLLHVIVGM
>ZP_01723236_TM_#03
FAFKDFIDDHLFKVEHVVYA
>ZP_01723236_TM_#04
KPKVDSLDKISYGLAF
>ZP_01723236_TM_#05
LVSVLKNWDTLSMDYFGFYAVGFISSFI
>ZP_01723236_TM_#06
ALISKVKLM
>AAA25717_TM_#01
MHLTITYWIDRLREAYPHAVAILLKGSYARGEASAWSDIDFDVLVSDEEVEEYRTWIEPV
GERLVHISVAVEWVTGWERDSADPSSWSYGLPTQETTQLLWAADENIRRRLDRPFKVHPA
AEPEVEDTVEALGKIRNAMVRGDDLAVYQAAQVVGKLIPTLLVPINPPTYARFAREAIDR
ILAFPNVPEGFAADWLTCMGLVDRRTHDPQPTRPNEWCAARSRFCRRMRTSSVRISRGCW
KQDWYLRISART
>NP_880590_TM_#01
HTPGDAPGAADDTASDERA
>NP_880590_TM_#02
AHTLEQIS
>NP_880590_TM_#03
KQALGVGVAQC
>NP_880590_TM_#04
:
```

True Markers
at the top

ShortBRED markers

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Help

Junction/Quasi Markers at the bottom

```
SECS
>P14509_TM_#04
IEAGVVDVDDFDKEREGWTAEQVWEAMHRLLPLA
>P14509_TM_#05
LIVEGKVVGCIDVGRAGIADRYQDLAVLWNCLEEFEPSLQERLVAQYGIADPDRR
>1112175A_JM_#01__[1112175A_w=0.486,YP_001103000_w=0.143,YP_0011
03000_w=0.371]
LFEWEFVEKVDSAIMRLRRRAEPLLEGAALERYE
>1112175A_JM_#02__[1112175A_w=0.515,YP_001103000_w=0.333,YP_0011
03000_w=0.152]
RKYPRRRVEAAFDHAGVGGGAVVAYVRPEQWLRL
>ABF69686_JM_#01__[ABF69686_w=0.459,ABN80187_w=0.135,ZP_03989103
_w=0.405]
DTAYPGEIVILADDTLKLNDILGNEKLLPHKTRI
>NP_792892_QM33_#01__[NP_792892_w=0.500,YP_002081505_w=0.500]
PAAFISGLTGQFYKQFALTIAISTVISAFNSLT
>YP_002081505_JM_#01__[YP_002081505_w=0.630,NP_792892_w=0.370]
LGTIGGFRLQIEDRGNX
>YP_970399_JM_#01__[YP_970399_w=0.306,ZP_03552050_w=0.163,YP_997
055_w=0.163,YP_997055_w=0.102,CAJ93947_w=0.061,YP_001348697_w=0.
061,YP_316450_w=0.041,YP_002092118_w=0.061,Q2KX31_w=0.041]
GGMLLGLSRKAATDX
>ZP_01817983_JM_#01__[ZP_01817983_w=0.493,YP_001694417_w=0.362,Y
P_001694417_w=0.145]
TLTGPFIGGFIKEDFQPVAKEKAIPTKELFTSVK
(END)
```

Running ShortBRED-Quantify

- Using your existing HMP data subset, you can search for antibiotic resistance proteins in the oral cavity by running:

```
shortbred_quantify.py
    --markers ardb_markers.faa
    --wgs 763577454-SRS014472-Buccal_mucosa.fasta
    --results 763577454-SRS014472-Buccal_mucosa-ARDB.txt
```

  - This should just a few seconds
  - It will again produce lots of status output as it runs

```
less 763577454-SRS014472-Buccal_mucosa-ARDB.txt
```

ShortBRED marker quantification

ubuntu@ip-10-170-15-59: ~/galeb

File Edit View Search Terminal Help

Family	Count	Hits	TotMarkerLength
YP_001694417	2380.9523809523807	1	26
ZP_04679156	0.0	0	235
ZP_04657259	0.0	0	136
ZP_04635798	0.0	0	91
ZP_04635523	0.0	0	171
ZP_04633951	0.0	0	59
ZP_04616832	0.0	0	9
ZP_04613685	0.0	0	72
ZP_04606269	0.0	0	183
ZP_04577926	0.0	0	168
ZP_04543635	0.0	0	173
ZP_04543532	0.0	0	186
ZP_04433866	0.0	0	187
ZP_04431003	0.0	0	95
ZP_04405580	0.0	0	169
ZP_04405450	0.0	0	300
ZP_04309403	0.0	0	138
ZP_04284182	0.0	0	177
ZP_04244950	0.0	0	51
ZP_04210257	0.0	0	113
ZP_04197552	0.0	0	129
ZP_04175489	0.0	0	70
ZP_04174269	0.0	0	21
ZP_04151022	0.0	0	27

:

RPKMs and raw hit count

Other columns are family name and AA marker length

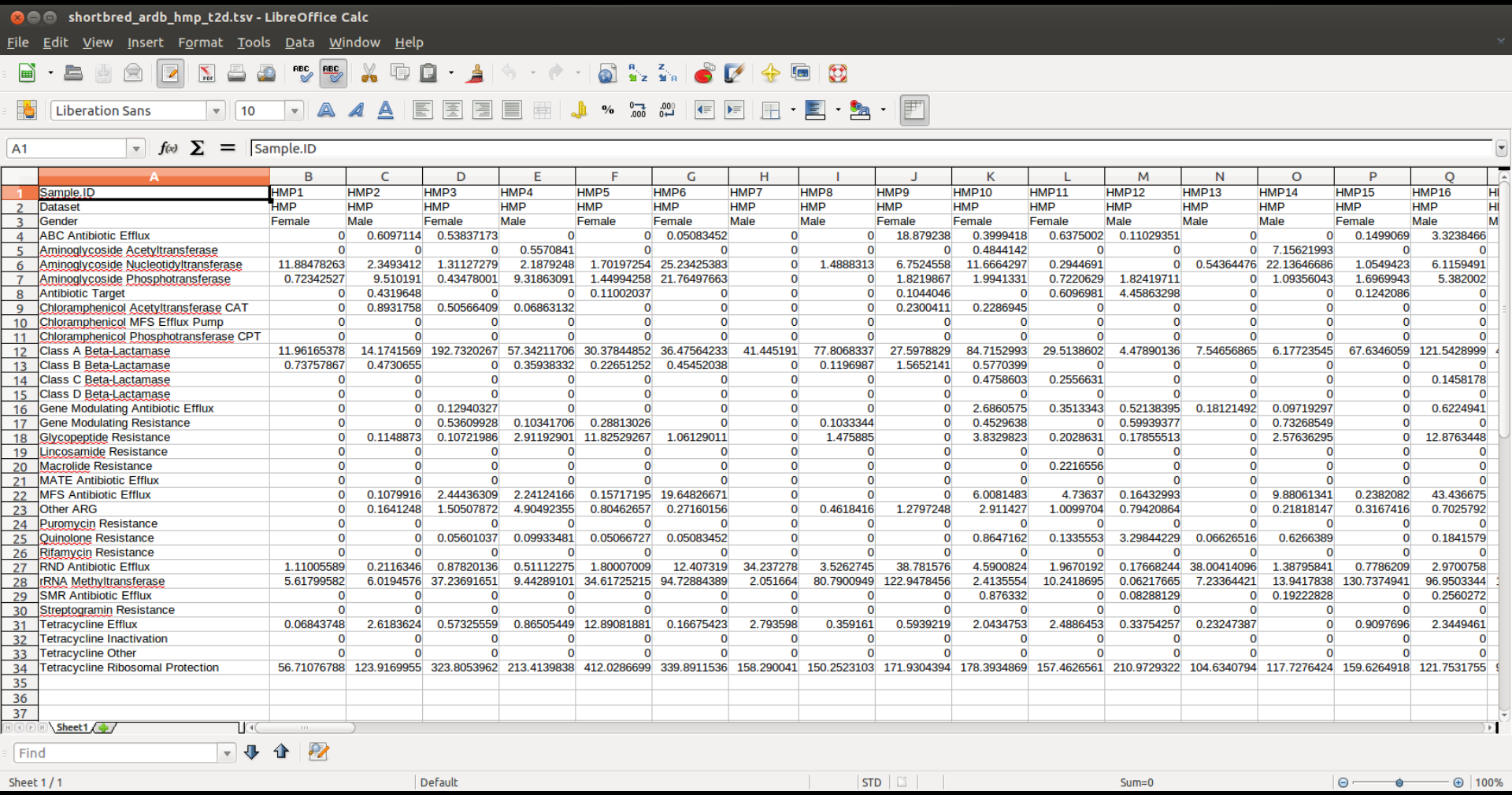
AR proteins in the human gut

- That's boring! Let's get some real data
- `cp` this file to your own working directory:

```
/home/ubuntu/biobakery/shortbred/data/shortbred_ardb_hmp_t2d.tsv
```

- This is the result of running:
  - ShortBRED-Identify on the real ARDB + reference
  - ShortBRED-Quantify on the real HMP + T2D data (Qin Nature 2014, PMID: 25079328)
  - Summing each sample's RPKMs for families in each ARDB resistance class

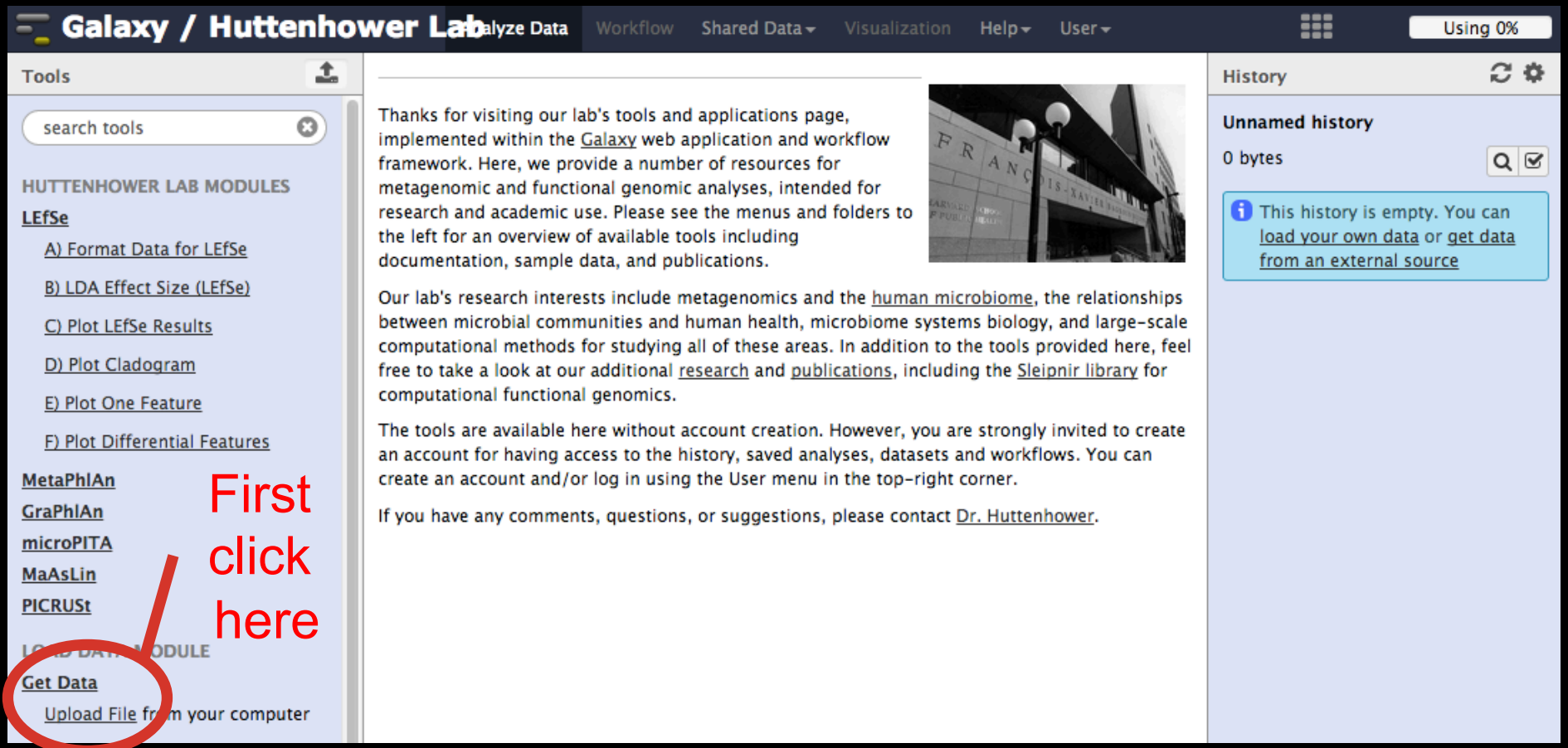
AR proteins in the human gut



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Sample.ID	HMP1	HMP2	HMP3	HMP4	HMP5	HMP6	HMP7	HMP8	HMP9	HMP10	HMP11	HMP12	HMP13	HMP14	HMP15	HMP16
2	Dataset	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP	HMP
3	Gender	Female	Male	Female	Male	Female	Female	Male	Male	Female	Female	Female	Male	Male	Male	Female	Male
4	ABC Antibiotic Efflux	0	0.6097114	0.53837173	0	0	0.05083452	0	0	18.879238	0.3999418	0.6375002	0.11029351	0	0	0.1499069	3.3238466
5	Aminoglycoside Acetyltransferase	0	0	0	0.5570841	0	0	0	0	0	0.4844142	0	0	0	7.15621993	0	0
6	Aminoglycoside Nucleotidyltransferase	11.88478263	2.3493412	1.31127279	2.1879248	1.70197254	25.23425383	0	1.4888313	6.7524558	11.6664297	0.2944691	0	0.54364476	22.13646686	1.0549423	6.1159491
7	Aminoglycoside Phosphotransferase	0.72342527	9.510191	0.43478001	9.31863091	1.44994258	21.76497663	0	0	1.8219867	1.9941331	0.7220629	1.82419711	0	1.09356043	1.6969943	5.382002
8	Antibiotic Target	0	0.4319648	0	0	0.11002037	0	0	0	0.1044046	0	0.6096981	4.45863298	0	0	0.1242086	0
9	Chloramphenicol Acetyltransferase CAT	0	0.8931758	0.50566409	0.06863132	0	0	0	0	0.2300411	0.2286945	0	0	0	0	0	0
10	Chloramphenicol MFS Efflux Pump	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	Chloramphenicol Phosphotransferase CPT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	Class A Beta-Lactamase	11.96165378	14.1741569	192.7320267	57.34211706	30.37844852	36.47564233	41.445191	77.8068337	27.5978829	84.7152993	29.5138602	4.47890136	7.54656865	6.17723545	67.6346059	121.5428999
13	Class B Beta-Lactamase	0.73757867	0.4730655	0	0.35938332	0.22651252	0.45452038	0	0.1196987	1.5652141	0.5770399	0	0	0	0	0	0
14	Class C Beta-Lactamase	0	0	0	0	0	0	0	0	0	0.4758603	0.2556631	0	0	0	0	0.1458178
15	Class D Beta-Lactamase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	Gene Modulating Antibiotic Efflux	0	0	0.12940327	0	0	0	0	0	0	2.6860575	0.3513343	0.52138395	0.18121492	0.09719297	0	0.6224941
17	Gene Modulating Resistance	0	0	0.53609928	0.10341706	0.28813026	0	0	0.1033344	0	0.4529638	0	0.59939377	0	0.73268549	0	0
18	Glycopeptide Resistance	0	0.1148873	0.10721986	2.91192901	11.82529267	1.06129011	0	1.475885	0	3.8329823	0.2028631	0.17855513	0	2.57636295	0	12.8763448
19	Lincosamide Resistance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	Macrolide Resistance	0	0	0	0	0	0	0	0	0	0	0.2216556	0	0	0	0	0
21	MATE Antibiotic Efflux	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	MFS Antibiotic Efflux	0	0.1079916	2.44436309	2.24124166	0.15717195	19.64826671	0	0	0	6.0081483	4.73637	0.16432993	0	9.88061341	0.2382082	43.436675
23	Other ARG	0	0.1641248	1.50507872	4.90492355	0.80462657	0.27160156	0	0.4618416	1.2797248	2.911427	1.0099704	0.79420864	0	0.21818147	0.3167416	0.7025792
24	Puromycin Resistance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	Quinolone Resistance	0	0	0.05601037	0.09933481	0.05066727	0.05083452	0	0	0	0.8647162	0.1335553	3.29844229	0.06626516	0.6266389	0	0.1841579
26	Rifamycin Resistance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	RND Antibiotic Efflux	1.11005589	0.2116346	0.87820136	0.51112275	1.80007009	12.407319	34.237278	3.5262745	38.781576	4.5900824	1.9670192	0.17668244	38.00414096	1.38795841	0.7786209	2.9700758
28	rRNA Methyltransferase	5.61799582	6.0194576	37.23691651	9.44289101	34.61725215	94.72884389	2.051664	80.7900949	122.9478456	2.4135554	10.2418695	0.06217665	7.23364421	13.9417838	130.7374941	96.9503344
29	SMR Antibiotic Efflux	0	0	0	0	0	0	0	0	0	0.876332	0	0.08288129	0	0.19222828	0	0.2560272
30	Streptogramin Resistance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	Tetracycline Efflux	0.06843748	2.6183624	0.57325559	0.86505449	12.89081881	0.16675423	2.793598	0.359161	0.5939219	2.0434753	2.4886453	0.33754257	0.23247387	0	0.9097696	2.3449461
32	Tetracycline Inactivation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	Tetracycline Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	Tetracycline Ribosomal Protection	56.71076788	123.9169955	323.8053962	213.4139838	412.0286699	339.8911536	158.290041	150.2523103	171.9304394	178.3934869	157.4626561	210.9729322	104.6340794	117.7276424	159.6264918	121.7531755
35																	
36																	
37																	

What it means: LEfSe

- Visit LEfSe at: http://huttenhower.sph.harvard.edu/lefse

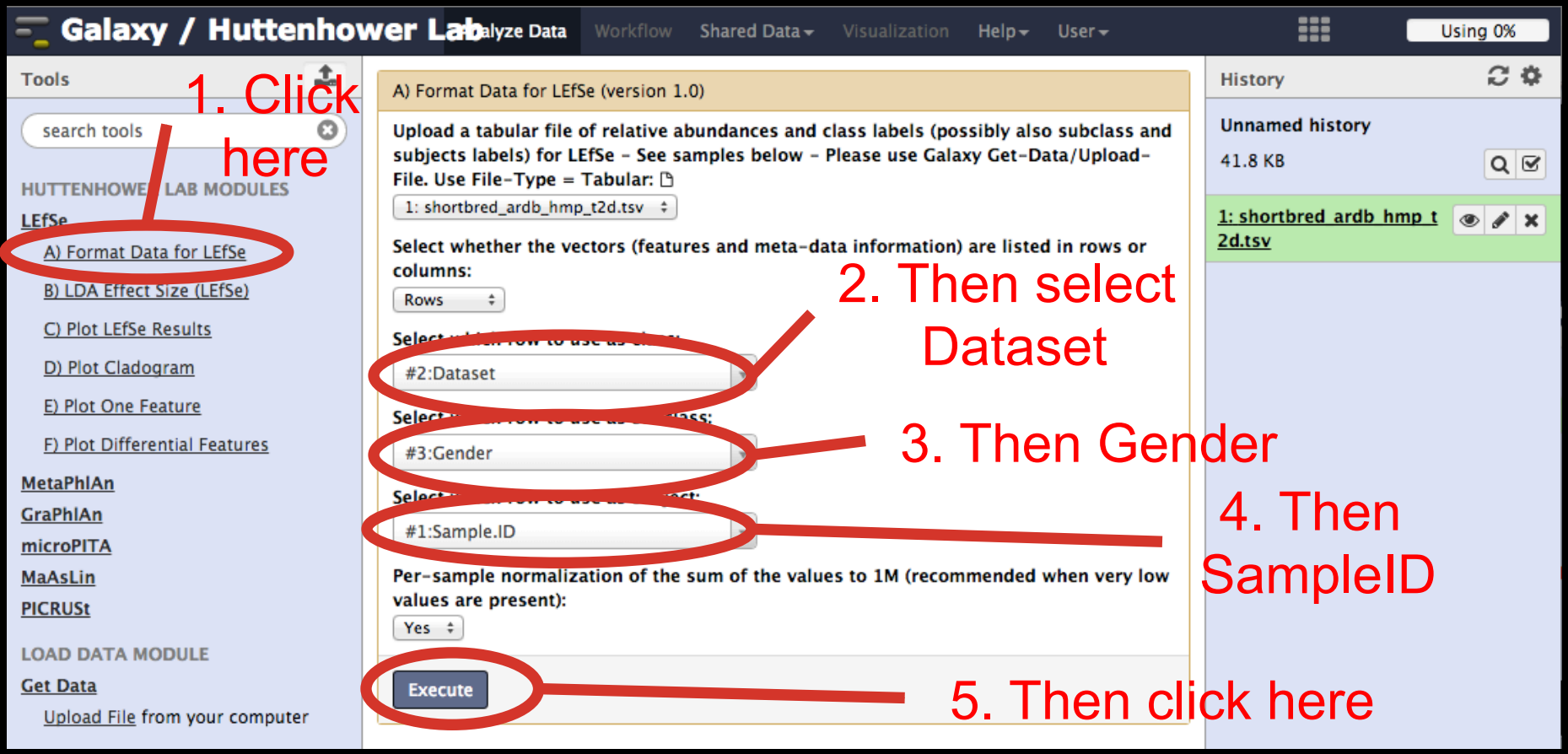


What it means: LEfSe

- Then upload your formatted table
  - After you upload, wait for the progress meter to turn green!

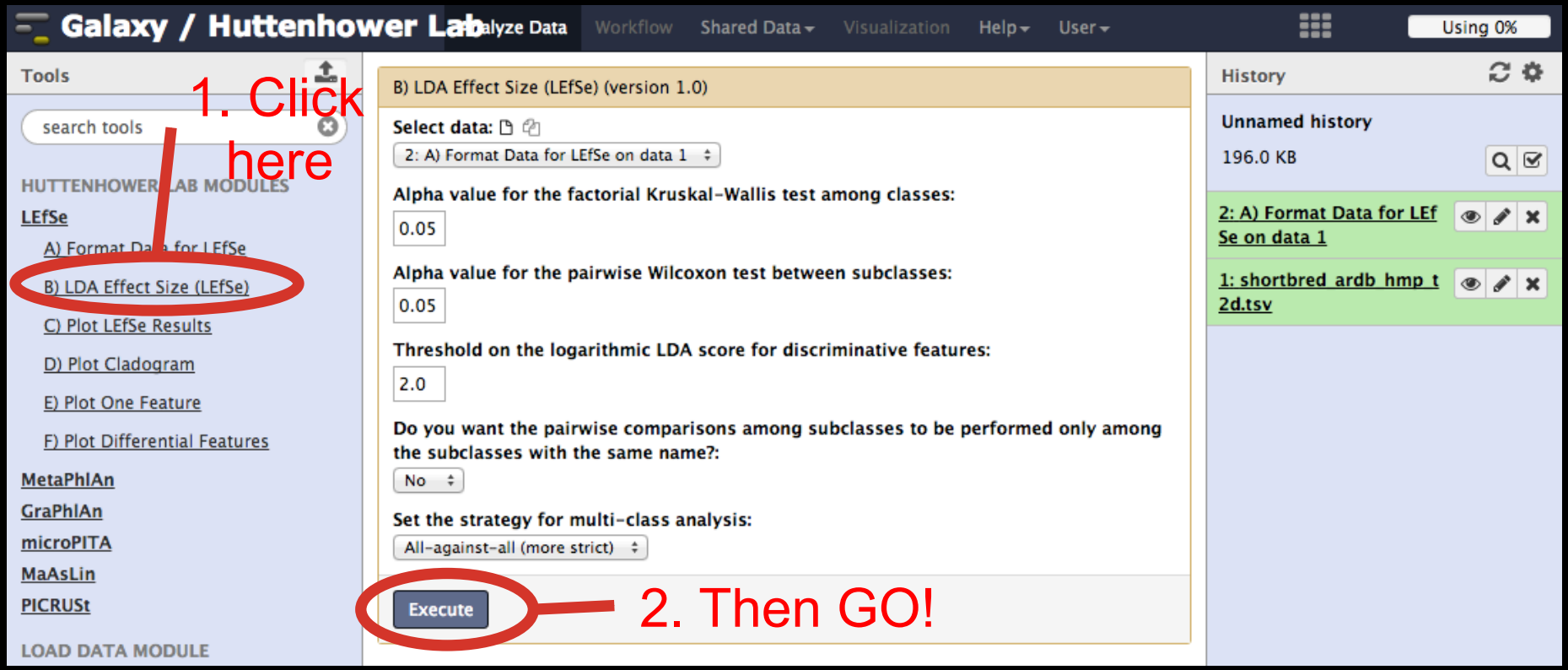
What it means: LEfSe

- Then tell LEfSe about your metadata:



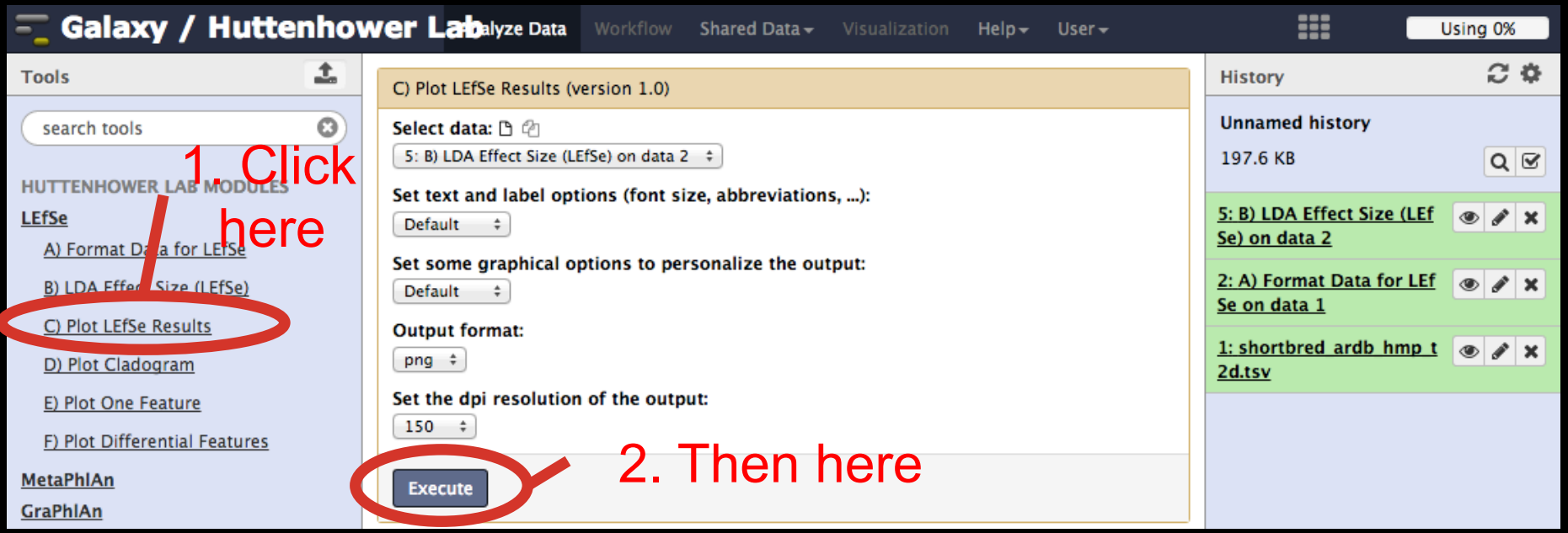
What it means: LEfSe

- Leave all parameters on defaults, and run LEfSe!
  - You can try playing around with these parameters if desired



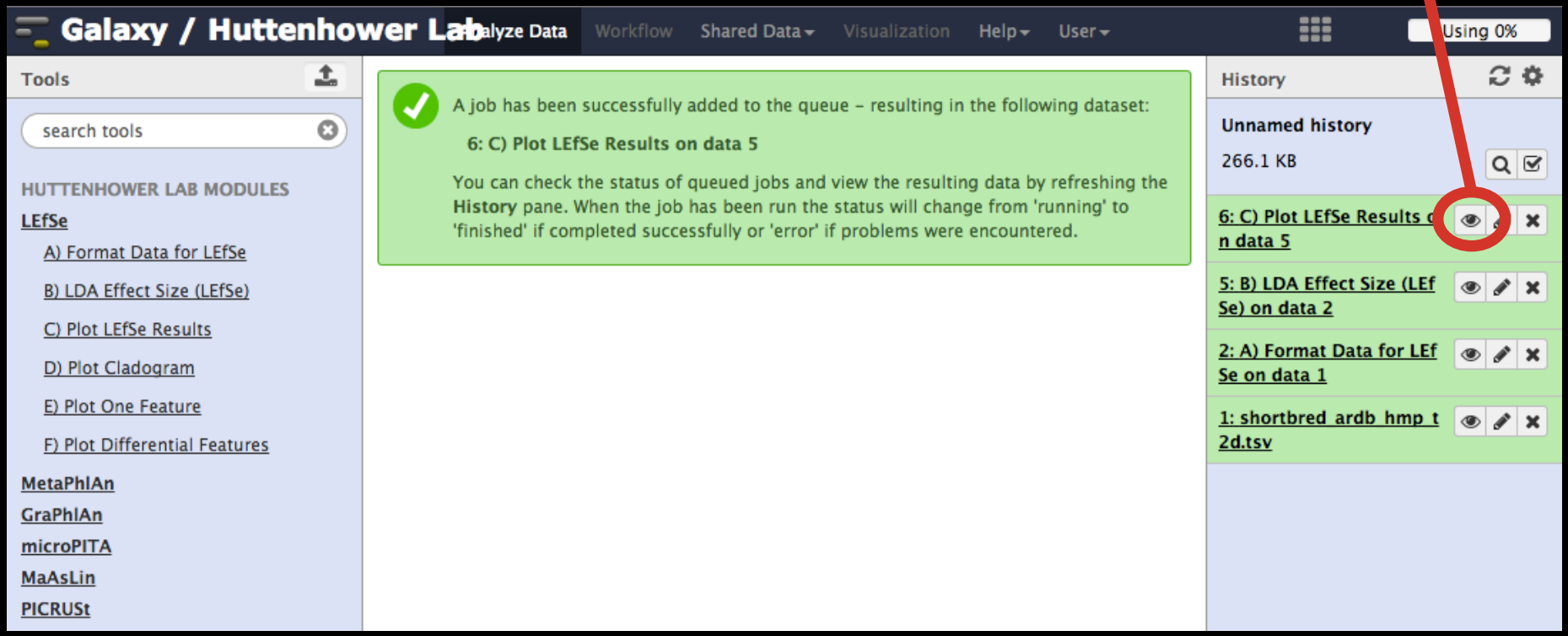
What it means: LEfSe

- You can plot the results as a bar plot
  - Again, lots of graphical parameters to modify if desired

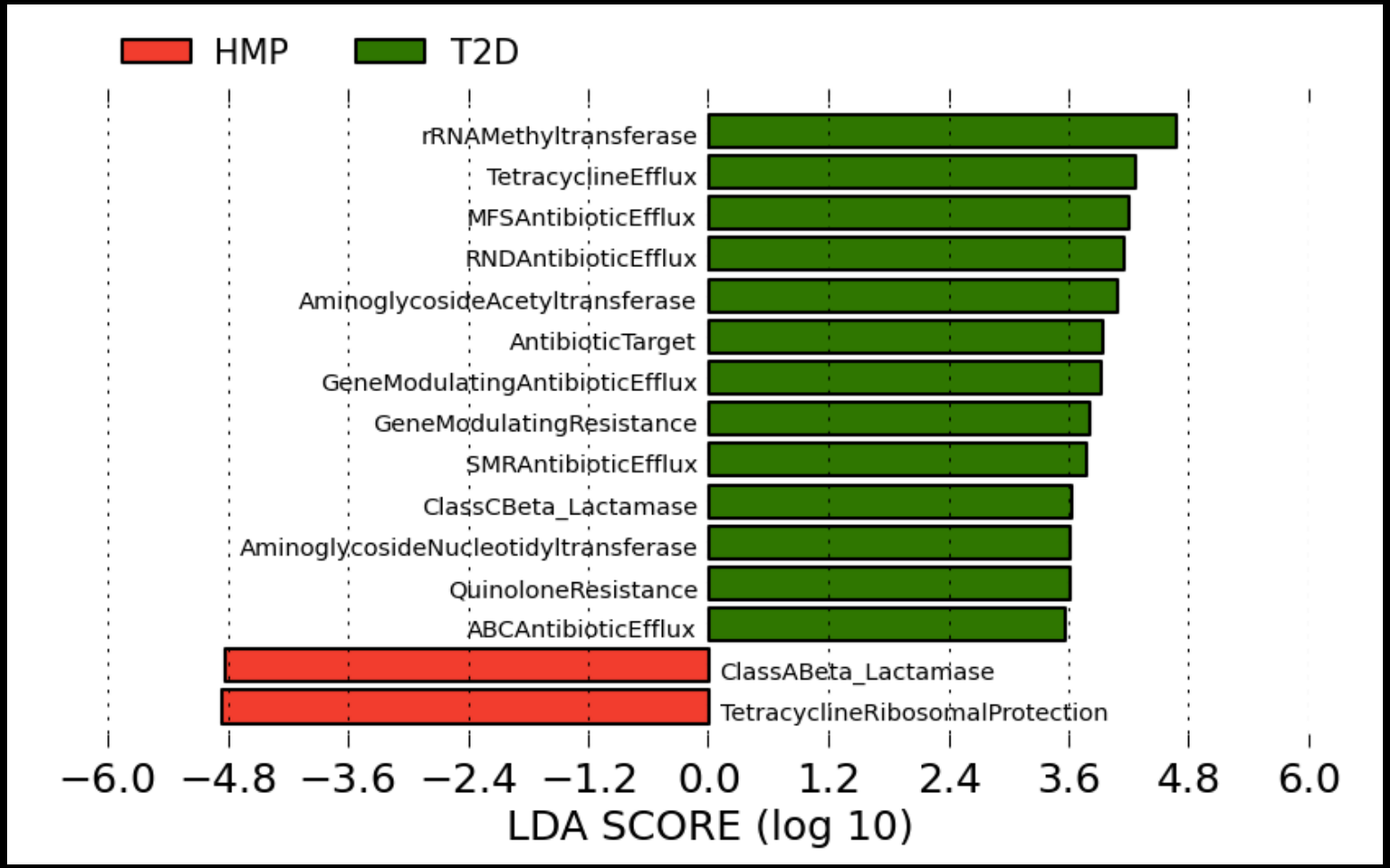


What it means: LEfSe

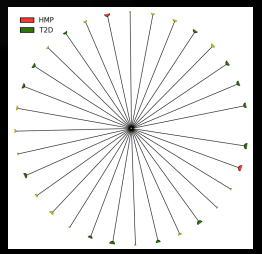
- In Galaxy, view a result by clicking on its “eye”



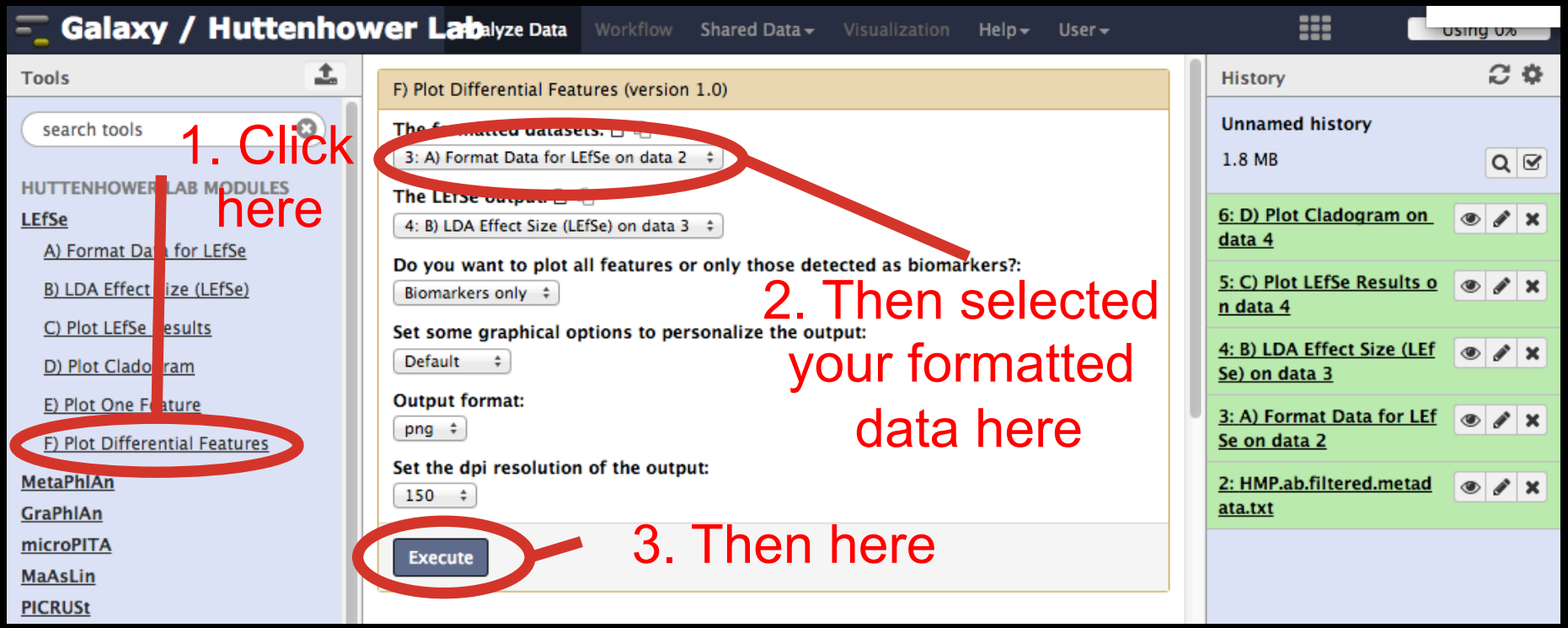
What it means: LEfSe



What it means: LEfSe

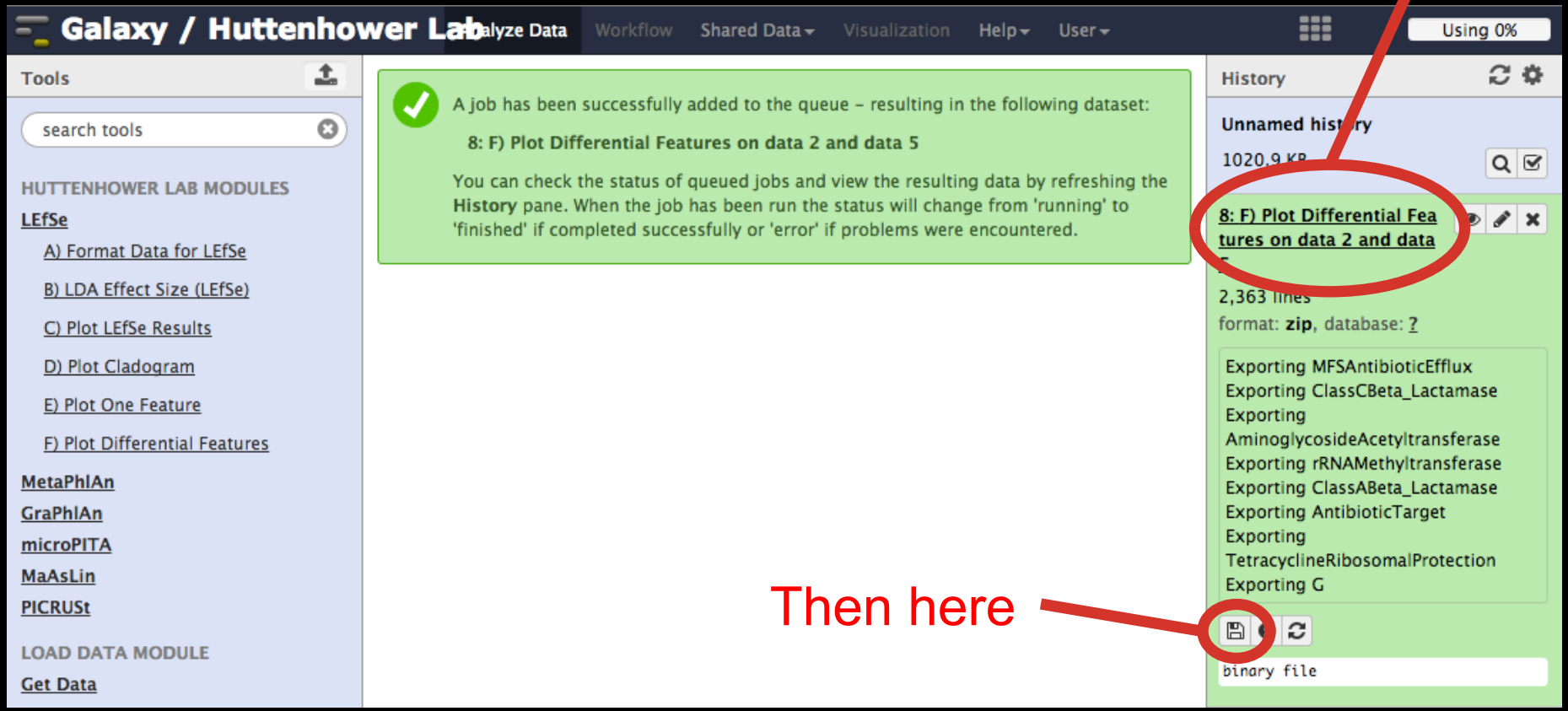


- There's no really any reason to plot a cladogram
  - Although it will work!
- But you can see the raw data for individual biomarkers
  - These are generated as a zip file of individual plots



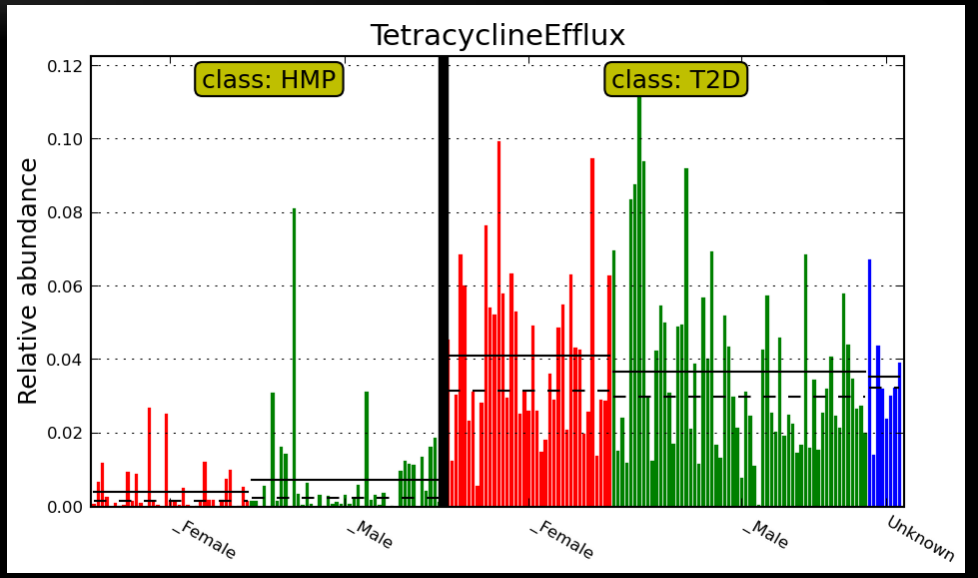
What it means: LEfSe

- In Galaxy, download a result by clicking on its “disk”

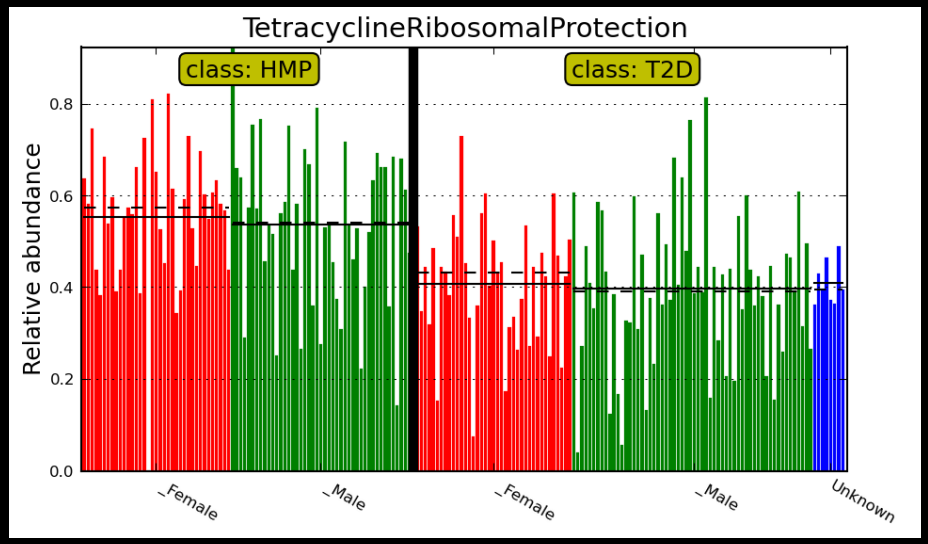


What it means: LEfSe

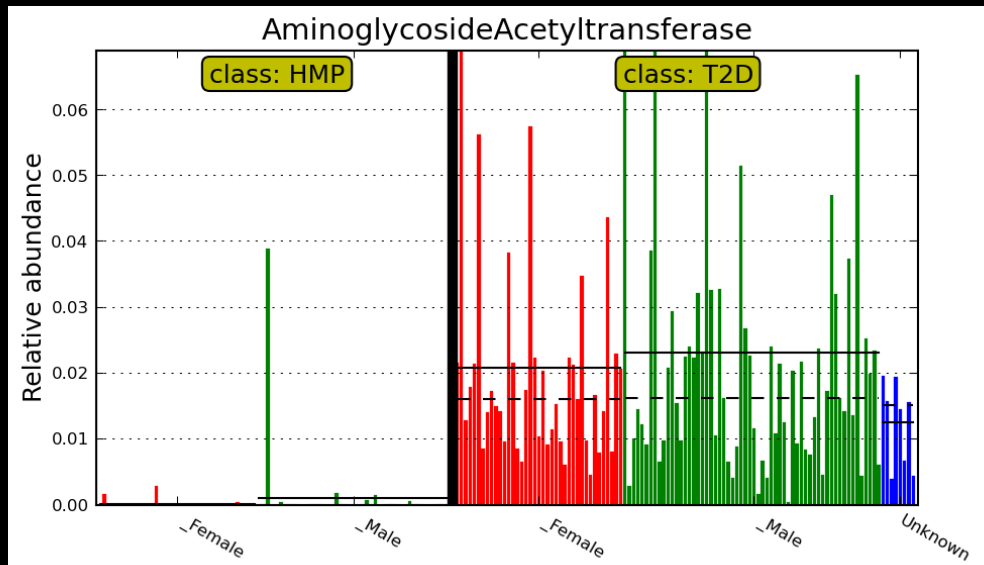
Tetracycline Efflux Pumps



Tet. Ribosomal Blockers



Aminoglycoside Acetyltransferases



Summary

- ShortBRED
  - Raw metagenomic reads,
    Proteins of interest, and
    Protein reference database in
  - Tab-delimited gene family rel. abundances out

http://huttenhower.sph.harvard.edu/biobakery

Huttenhower Lab Tools

Welcome to the official Huttenhower Tutorials wiki.

We now support `bioBakery`, a virtual environment platform that provides Huttenhower tools (already installed!). Please click on the button below for more information:

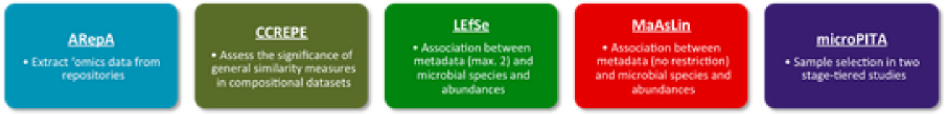
The wiki provides tutorials for Huttenhower tools, illustrating through demos how to use these tools on your datasets. Huttenhower tools can be divided under three main categories as shown below. Click on the tool for the corresponding tutorial.

Composition Analysis

These tools can determine the composition in terms of (i) microbial species and their associated abundances (MetaPhlAn) or (ii) genes and associated pathways (HUMAnN) in the dataset. Please click on the links below for detailed tutorials:

Statistical Analysis

These tools can determine the associations from the provided metadata information and microbial composition tables. Please click on the links below for detailed tutorials:

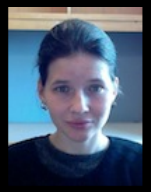


Visualization

These tools can help visualize taxonomical and phylogenetic information for (i) microbial composition/taxonomy data, (ii) outputs from MetaPhlAn, LEfSe, HUMAnN, MaAsLin. Please click on the link below for detailed tutorial:

Thank you!


Curtis Huttenhower


Xochitl Morgan


Afrah Shafquat

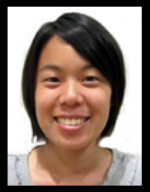

Keith Bayer


George Weingart


Regina Joice


Aleksandar Kostic


Chengwei Luo

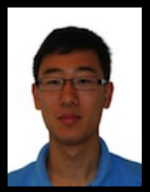

Tiffany Hsu


Emma Schwager


Koji Yasuda


Kevin Oh


Boyu Ren


Andy Shi


Jim Kaminski


Joseph Moon


Randall Schwager


Levi Waldron


Nicola Segata


Wendy Garrett
Michelle Rooks

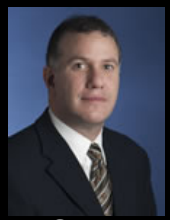
Ruth Ley
Omry Koren


Dirk Gevers
Kat Huang


Ramnik Xavier
Harry Sokol
Dan Knights
Moran Yassour


Rob Beiko
Morgan Langille


Rob Knight
Greg Caporaso
Jesse Zaneveld


Mark Silverberg
Boyko Kabakchiev
Andrea Tyler

Jacques Izard
Katherine Lemon

Bruce Sands

Human Microbiome Project

Owen White
Joe Petrosino
George Weinstock
Karen Nelson
Lita Proctor
Erica Sodergren
Anthony Fodor
Marty Blaser
Jacques Ravel
Pat Schloss
Sahar Abubucker
Brandi Cantarel
Alyx Schubert
Mathangi Thiagarajan
Beltran Rodriguez-Mueller
Makedonka Mitreva
Yuzhen Ye
Mihai Pop
Larry Forney
Barbara Methe

Bruce Birren Mark Daly
Doyle Ward Ashlee Earl